

Anatomical, developmental and physiological aspects of silica in wheat

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**ANATOMICAL, DEVELOPMENTAL AND PHYSIOLOGICAL  
ASPECTS OF SILICA IN WHEAT**

**HELEN J. TUBB**

**A thesis submitted in partial fulfilment of the requirements of Oxford  
Brookes University for the degree of Doctor of Philosophy.**

**April 1995.**

## ABSTRACT

The major aims of this thesis were to study silica deposits in cereal inflorescences and wheat roots and investigate soluble/deposited silica partitioning and uptake of silica by wheat.

The morphology of a particular phytolith (the papilla) from inflorescence bracts of 70 species from *Triticum* (wheat), *Hordeum*, *Aegilops* families and *Secale cereale* was analyzed. Using papilla pit number and/or diameter it is possible to distinguish between *Hordeum* sp. and *Triticum* sp., and between wheats of different ploidy levels.

The root growth of *T. aestivum* was reduced by silica in the nutrient solution (Si+) during the first six days of growth, compared to plants grown in solution not supplemented by silica (Si-). After a further four days Si+ plants had significantly longer roots than Si- plants. At the light microscope level, a time course for silicification of the inner tangential wall of the root endodermis of *T. aestivum* was elucidated over ten days. Very few walls were silicified after 24 hours exposure to Si+ solution, but by day 10 up to 80% of endodermal cells in the basal area of the root were silicified. The percentage of silicified cells increased from the apex to the base of the root. Silica in *T. aestivum* was measured quantitatively using a molybdate blue detection method. Typically, 99% of the total silica in the seedlings was in the deposited form. The average concentration of soluble silica within the xylem exudate was 3.6 mM. The uptake of silica was affected by the silica concentration and pH of the nutrient solution. By measuring transpirational water loss and the silica content of the plants, it was concluded that silica uptake was active in *T. aestivum*.

Uptake mechanisms were investigated by growing seedlings in nutrient solutions containing 2 mM Si and an inhibitor for 24 hours. The inhibitors used were ATPase inhibitors (sodium orthovanadate, diethylstilbestrol, erythrosin B) and ionophores (nigericin, FCCP). The data suggests that functional ATPases and a proton gradient are required for silica uptake. Transport into the xylem and uptake at the root surface may be differentially affected by the inhibitors.

Some of the results presented in this thesis have been presented/published in the following.

Tubb HJ & Hodson MJ. (1994). Early stages of silica deposition in wheat roots. Poster presented at "Biomineralisation: The relevance of the geological, chemical and biological interface", The Nottingham Trent University.

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*"Imagination is More Important Than Knowledge"*

*Albert Einstein.*

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## LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
APS	3-aminopropyltriethoxysilane
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphate hydrolase
CVL	Crystal violet lactone
DES	Diethylstilbestrol
DWT	Dry weight
EB	Erythrosin B
FCCP	carbonyl cyanide p-trifluoromethoxyphenylhydrazone
FITC	Fluorescein isothiocyanate
FWT	Fresh weight
ICS	Intercellular spaces
ITW	Inner tangential wall
LM	Light microscope
MR	Methyl red
PM	Plasma membrane
ppm	Parts per million
PSU	Primary spherical unit
R.I.	Refractive index
SEM	Scanning electron microscope
Si+	Full nutrient solution supplemented with silica in the form of sodium metasilicate
Si-	Full nutrient solution without additional silica
Std.	Standard deviation
TCA	Trichloroacetic acid
TEM	Transmission electron microscope
<i>Triticum</i> sp.	
AA	<i>Triticum</i> species with the genetic designation AA
AABB	<i>Triticum</i> species with the genetic designation AABB
AABBDD	<i>Triticum</i> species with the genetic designation AABBDD
AAGG	<i>Triticum</i> species with the genetic designation AAGG
AAAAGG	<i>Triticum</i> species with the genetic designation AAAAGG
TSCF	Transpiration stream concentration factor
XPC	Xylem parenchyma cells

## CHAPTER 1: GENERAL INTRODUCTION.

### **1.1. Introduction.**

Silicon ( $\text{Si}^{14}$ ) is a nonmetallic element in group four of the periodic table, positioned below carbon ( $\text{C}^6$ ) which is the basis for life on earth. In contrast, the importance of silicon for life remains unclear.

The chemistry of carbon and silicon shows many differences, even though both elements form four covalent bonds. Silicon is a larger atom than carbon, forms ionic bonds when combined with strongly electronegative elements, and in the presence of water undergoes hydrolysis. Carbon, by comparison, remains unaffected by water, and rarely forms ionic bonds. The building of compounds provides one further difference, with silicon typically forming Si-oxygen-Si bonds rather than the simple C-C bonds of carbon (Sosman, 1969).

Chemical properties aside, however, silicon is the second most abundant element on earth, with oxygen being the first (Greenwood & Earnshaw, 1984). Elemental silicon does not occur naturally, but is usually bound to oxygen (O) or oxygen and other elements.

Silica is silicon dioxide (silicon (IV) oxide) and is the major constituent of over 95% of rocks on earth. It is found in three principal crystalline forms; quartz, tridymite and cristobalite (the latter two are relatively rare minerals). In addition, coloured quartz minerals include amethyst (a purple/violet colour), blue quartz and rose quartz. Alternatively, silica can occur in a non-crystalline form, which is termed amorphous. Opal is hydrated amorphous silica which typically contains 1-21% water and has the chemical formula  $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ . Precious opal gemstones are typified by coloured surface reflections caused by the interference of light along minute cracks and other internal inhomogeneities within the silica. The milkiness of white opals is caused by a large number of tiny gas filled cavities trapped within the stone (Jahns, 1969).

It is the amorphous form of silica that has been detected in plants, and although chemically similar to precious opal, it does not have the same appearance and is termed biogenic opal. The most famous examples of

biogenic opal are the silicified valves of the diatoms. These are single-celled algae which have their cell wall surrounded by silica, and to these organisms, silica is an essential element for life.

Silica occurs, at varying abundance, throughout higher plant species. Much of the work on silica within plants has been directed towards deposited biogenic opal because it is very resilient to chemical and heat attack and, therefore, easy to extract using destructive methods. The history of deposited silica studies in plants has been reviewed by Powers (1992). The highest levels of silica accumulation, typically, occur in the aerial parts of the plant, especially inflorescence bracts of grasses. Lower levels of silica occur in the leaves and the lowest levels in the roots. The silica deposits which develop in the aerial organs of plants, and their possible usage in prehistoric studies, is reviewed in chapter 2. The subterranean silica deposits and possible deposition mechanisms (of both aerial and subterranean deposits) are reviewed in chapter 4. No higher plant has been shown to have an absolute nutritional requirement for silica (except possibly *Equisetum* species and rice), and at best silicon is generally classified as a beneficial element for plant growth (Epstein, 1994). The potential roles of silica in plant nutrition and growth are examined in chapter 3.

As silica is a major component of rocks, it is present in soils and is thought to occur as soluble monosilicic acid in soil solution. Plants growing in the soil are exposed to silica in solution at the root surface. Some of this silica is transported to the aerial parts of plants, with the xylem vessels being the most likely candidate for silica transport within the plant. The majority of silica within a plant is deposited either as intercellular and/or intracellular deposits. The partitioning of silica within plants is examined in chapter 4, together with a review of possible deposition mechanisms.

The mechanisms of silica uptake in higher plants are unclear. It is thought to be taken up as monosilicic acid which is a very weak acid at physiological pH and predominately uncharged. For many dicotyledonous plants it appears silica enters the plant roots passively, being taken up with

water in the transpiration stream. Many monocotyledonous plants however, display active uptake mechanisms. The possible methods of silica uptake in plants are reviewed in chapter 5.

## 1.2. Aims of the thesis.

The first area of work in this study, chapter 2, is focused on the silica deposits (termed phytoliths) which occur within the inflorescence bracts of cereals. The size, shape and form of these deposits may have taxonomical and archaeological significance, and this area of study is concerned with producing a database for the measurements of a particular phytolith (the papilla). This database aims to distinguish between barley, wheat and wheat progenitors on the basis of the papilla parameters alone and to identify possible overlaps in papilla morphology within the group.

The second area of study, chapter 3, is concerned with the effect of silica in a full nutrient solution on the growth of young wheat (*Triticum aestivum* L. cv. Beaver) plants over a 10 day growth period. Silica is known to have beneficial effects on plant growth, and this particular area of study is directed towards determining the effect on growth parameters during the early stages of growth in wheat.

The following area of research is focused on the development of silica deposits within the roots of young wheat seedlings (chapter 4). The deposits occur within the cell walls of the endodermis, and this investigation aims to elucidate a time course, over 10 days, for this specific deposition. Possible techniques for the detection of silica within wheat plants after a 24 hour exposure to a silica enriched nutrient solution are also investigated. One further aim is to investigate the quantity and partitioning of soluble and deposited silica within the wheat plant, and measure any variations which may occur during a short growth period of 10 days.

The precise nature of silica uptake mechanisms in higher plants remains elusive. This final area of research is approached in chapter 5, and involves the investigation of possible uptake mechanisms in wheat over a 24 hour time period. This chapter is a preliminary investigation examining

the possible uptake mechanisms using specific inhibitors to study silica uptake and transport within the young wheat plants.

## CHAPTER 2: PHYTOLITH STUDIES.

### **2.1. Introduction.**

Phytolith translated literally means "plant stone" and it is usually applied to "stones" comprising of amorphous silica. Phytoliths are produced within living plants, and this is in contrast to silicified fossils, where deposition occurs after death. The deposition process and possible functions of silica deposition in plants will be reviewed in chapter 4.

Silica deposits can form either intracellularly or intercellularly depending on the plant organ and species. Intracellular deposits fill the cell lumen with amorphous silica such that the phytolith resembles the original cell in detail. Intercellular deposits form within the cell wall matrix and between cells, exterior to living protoplasts. Many plants have been shown to produce phytoliths (for examples see table. 2.1). Phytoliths can occur in all organs of the plant, although the majority are found within the aerial structures. For example the inflorescence bracts of grasses frequently contain up to 20 % of the dry weight as silica (Van Soest, 1970).

Phytoliths are very resilient to chemical and heat attack, and thus when a plant dies, although the organic matter decomposes, the phytoliths remain unaltered in the soil where the plant died, remaining as an almost permanent record of the plant. For example, radiocarbon dating of occluded carbon within phytoliths extracted from soil samples from west-central Ohio demonstrated that they were produced  $13,300 \pm 450$  years before present (Wilding, 1967). Their resilience has led to phytoliths being classified as microfossils, along with pollen, and having possible archaeological applications (reviewed by Piperno & Pearsall, 1993), especially in palaeobotany and paleoenvironmental reconstruction.

Phytolith analysis is similar to palynology (pollen studies), as both aim to identify plants from microscopic remains. The historic development of both types of study has been reviewed by Bryant (1993). Although pollen is advantageous as it is species specific, phytoliths are more resilient than pollen under certain environmental conditions. This aspect of phytoliths is particularly useful in determining lifestyles of early cultures, as



**Table 2.1.** Examples of Investigations of Aerial Silica Deposits in Plants

Species	Organ	Reference
<i>Avena sativa</i>	Leaf, internodes, inflorescence bracts	Baker (1960), Kaufman <i>et al.</i> (1969), Soni, Kaufman & Bigelow (1970), Kaufman <i>et al.</i> (1972)
British grasses & Cereals	Inflorescence bracts	Parry & Smithson (1966)
Deciduous Angiosperm Trees (36 Species)	Leaves	Geis (1973)
<i>Dichanthelium</i> (11 Species)	Paleas	Clark & Gould (1975)
<i>Echinochloa</i> sp.	Paleas	Clark & Gould (1975)
<i>Equisetum</i> sp.	Stem epidermis	Kaufman <i>et al.</i> (1971)
<i>Ficus lyrata</i>	Leaf cuticle	Davis (1987)
<i>Hordeum vulgare</i>	Nodes, leaves, culms and inflorescence bracts	Hayward & Parry (1973, 1975, 1980)
<i>Lolium multiflorum</i>	Leaf mesophyll	Dinsdale, Gordon & George (1979)
<i>Magnolia grandiflora</i>	Leaves	Postek (1981)
<i>Oryza sativa</i>	Inflorescence bracts	Soni & Parry (1973)

continued overleaf

Table 2.1. continued.

<i>Panicum</i> (10 Species)	Paleas	Clark & Gould (1975)
<i>Phalaris canariensis</i>	Lemmas, glumes Inflorescence bracts	Hodson, Sangster & Parry (1984,1985), Sangster <i>et al.</i> (1983)
<i>Pteridium aquilinum</i>	Fronds	Parry, Hodson, Newman (1985)
<i>Setaria italica</i>	Caryopsis and inflorescence bracts	Parry & Hodson (1982)
<i>Triticum aestivum</i>	Inflorescence bract	Hodson & Sangster (1988)
<i>Urtica dioica</i>	Stinging emergences	Thurston (1974)
<i>Zizania</i> sp.	Lemmas, paleas and awns	Terrell & Wergin (1981)

phytoliths survive processes such as cooking and burning. Also, pollen is liberated in the general area, especially when considering wind-pollinators such as the grasses, whereas phytoliths remain where the plant died or where the plant remains were left by people. Further, phytoliths are abundant and distinctive in the Gramineae whereas pollen grains are most distinctive in trees (Mulholland & Rapp, 1985) and phytoliths may help subdivide the "Gramineae category" used by palynologists studying prairie and savannah areas (Rovner, 1971).

For phytoliths to be of any use for taxonomic or archaeological purposes they have to be extracted and classified. This classification system can then be used to identify the phytolith assemblage produced by specific plant families and possibly plant species.

#### Extraction of Phytoliths from Plants.

The morphology of the phytoliths can be examined extensively when they are removed from the plant material, and as phytoliths are resistant to chemical and heat attack, extraction from plant material is *via* destructive methods. A frequently used and simple method is dry-ashing (for example Twiss, Suess & Smith, 1969) in which dried plant material is ashed at about 500°C in a muffle furnace to burn off the organic matter. Morphologically the phytoliths appear undamaged by this method, although chemical changes may occur (Jones & Milne, 1963). An alternative option is to wet-ash the plant material using strong acids such as nitric, chromic, perchloric and sulphuric or a mixture, to remove the organic matter (Hayward & Parry, 1975, Kaplan, Smith & Sneddon, 1992). Theunissen (1994) used ashing, washing in hydrogen chloride, and sonication to remove impurities from phytoliths. Oxygen plasma has also been used to ash plant material and extract phytoliths (Dinsdale, Gordon & George, 1979). The above methods destroy the organic plant material completely and produce isolated phytoliths. However, various methods have been employed to produce "transparent" plant material using sodium hypochlorite (Parry & Smithson, 1958; Doolittle & Frederick, 1991; Kaplan, Smith & Sneddon, 1992) so that

when suitably mounted the phytoliths can be viewed as they were *in vivo*. Such preparations are termed spodograms.

#### Extraction of Phytoliths from Soil.

For archaeological samples it is frequently necessary to extract phytoliths from the soil. The method for extracting phytoliths from such samples was revised by Rovner (1983), based on a previously recommended procedure (Rovner, 1971). Initially the soil can be disaggregated using a commercial water softener such as "Calgon". Once this is completed the soil is rinsed and HCl is added to remove organic material which may cause aggregation of the soil particles. The sample is then centrifuged and dried. Separation of phytoliths from the soil separates is achieved by a flotation-sink method. A heavy liquid is produced with a specific gravity of 2.3, and typically this is a mixture of tetrabromoethane and absolute alcohol (Kalisz & Boettcher, 1990), bromoform/acetone (Rosen, 1989) or nitrobenzene/bromoform (Wilding, 1967). The phytoliths will float to the top of the liquid and can be removed and dried. Further steps including sedimentation and sieving can also be included (Kalisz & Boettcher 1990, Rosen, 1989, Wilding, 1967, Ollendorf, 1987). Once phytoliths have been extracted from the soil, various tests can be performed to determine the age of the phytolith (e.g. radiocarbon dating), although depth within the soil will give an indication. Also stable isotope ratio analysis (SIRA) has been applied to extracted phytoliths to aid in paleoenvironmental analysis (Fredlund, 1993).

#### Viewing Phytoliths.

Once the phytolith sample has been prepared (either as isolated phytoliths or a spodogram) by one of the above methods it can be viewed using light and/or electron microscopy. For light microscopy (LM), phytoliths can be visualized by mounting them in a suitable medium with a different refractive index (R.I.). Phytoliths have a R.I. of 1.43 (Parry & Smithson, 1958 [R.I.  $\approx$  1.42], Kaufman *et al.*, 1985) and various mounting

<sup>$\alpha$</sup>   
mediums have been used. Canada balsam has a R.I. of 1.52 (Parry & Smithson, 1958, Kiernan, 1981) and has been used extensively (for example see Parry & Smithson, 1966; Twiss, Suess & Smith, 1969; Parry & Hodson, 1982; Pearsall & Trimble, 1984; Doolittle & Frederick, 1991). Other mountants include Gurr's neutral mounting medium, (R.I.  $\approx$  1.51), cedarwood oil (R.I.  $\approx$  1.51 [Parry & Smithson, 1958]), Cadex, R.I. = 1.55 (Twiss, Suess & Smith, 1969) and Permunt in conjunction with Nomarski differential interference contrast microscopy (Ollendorf, 1987). Also, phytoliths display birefringence when exposed to polarized light microscopy (Parry & Smithson, 1958). This is not due, however, to the phytolith itself, as silica is amorphous, but is caused by silica deposition replicating the crystalline pattern of the cellulose fibrils (Kaufman *et al.*, 1985). Phytoliths can also be observed at the LM level using specific silica stains such as methyl red (MR), crystal violet lactone (CVL) and silver chromate (Dayanandan, Kaufman & Franklin, 1983). A further advancement to this technique was reported by Ball & Brotherson (1992) who analyzed phytoliths stained with MR and CVL using laser scanning microscopy.

In order to gain greater detail of phytolith morphology and 3-dimensional structure scanning electron microscopy (SEM), in secondary electron imaging (SEI) mode, has been employed (for example see Clark & Gould, 1975; Hayward & Parry, 1975, 1980), although the use of backscattered electron imaging (BEI) for visualizing superficial and subsuperficial phytoliths *in situ* in the plant tissue was proposed by Brandenburg *et al.* (1985). Transmission electron microscopy (TEM) has also been applied to the study of phytoliths within plant tissue (Kaufman *et al.*, 1985; Sakai & Thom, 1979). A further advancement in electron microscopy technique frequently used for phytolith detection is X-ray microanalysis with conventional electron microscopy (reviewed by Hodson & Sangster, 1990). This allows detection of the phytolith within prepared tissue, and confirmation that it is composed of silica, and numerous studies have used this method (for example Kaufman *et al.*, 1969; Soni, Kaufman & Bigelow 1970; Kaufman *et al.*, 1971; Kaufman *et al.*, 1972, Hayward &

Parry, 1973; Soni & Parry, 1973; Sakai & Thom, 1979; Terrell & Wergin, 1981; Parry & Hodson, 1982; Sangster *et al.*, 1983; Lanning & Eleuterius, 1992). Hodson & Sangster (1989a) have also used X-ray microanalysis in conjunction with BEI SEM techniques.

#### Classification of Phytoliths.

Once phytoliths have been extracted and viewed the next step is to classify them, and studies have, therefore, been focused towards phytolith classification in relation to the plant which produced the phytoliths. However, since many plants produce numerous forms of phytoliths this classification is extremely difficult. An important report was published by Twiss, Suess & Smith (1969), who divided phytoliths isolated from 17 grass species into 4 classes and 26 types. Three of the classes were specific to subfamilies of the grasses, Festucoid, Chloridoid and Panicoid. The fourth class, "Elongate phytoliths" was found in all 17 species studied. A "more detailed classification", based upon Twiss *et al.*, was developed by Mulholland & Rapp (1985) after examining 40 species. In this study 10 categories were proposed: dumbbell, cross, saddle, long trapezoid, short trapezoid, trichome, long cell, bulliform cell, stoma and rectangle/square. In a very extensive analysis, (comprising 17 domesticated and 365 wild species [55 different families] from the New World tropics), Piperno (1985) classified phytoliths according to the contributing plant tissue and the phytolith shape. More recently, Kaplan, Smith & Sneddon (1992) investigated 43 species of cereal and related graminaceous species and recognized 23 types of phytoliths.

#### The Uses of Phytolith Analysis.

The above examples illustrate the problems of producing a universal classification system for phytolith shapes. Despite the problems, research has continued to investigate the possibility that plant species/families could be identified from the phytolith assemblage that they produce. Indeed, in the study by Piperno (1985), squash appeared to produce a "unique"

phytolith when compared to the other species in the study, although a range of phytoliths was normally required to identify a particular family or species. The author stressed that other studies had to be conducted to examine possible overlaps of phytolith morphology.

#### Taxonomic Studies.

In taxonomic studies phytolith analysis has been successfully employed to distinguish between *Arundo donax* and *Phragmites communis* (Ollendorf, Mulholland & Rapp, 1988). These reed grasses are virtually indistinguishable from vegetative parts alone, but phytolith analysis demonstrated *A. donax* produced dumbbell and cross phytoliths and *P. communis* produced saddles and saddle-topped short trapezoids. It is also possible to differentiate between  $C_3$  and  $C_4$  plants due to their different incorporation of  $^{12}C:^{13}C$  ratio which is reflected in the occluded carbon within the phytolith (Kelly *et al.*, 1991). However, the potential use of phytolith analysis in taxonomic studies has yet to be exploited fully.

#### Prehistoric Studies.

The uses of phytolith analysis in prehistoric studies are quite varied. For example, analysis of soil phytoliths suggested the possible previous vegetation in a forest opening called Buffalo Beats in southeastern Ohio (Kalisz & Boettcher, 1990). Also, examination of phytoliths embedded in dental enamel of an extinct ape (*Gigantopithecus blacki*) led to the suggestion that it had a varied diet of grasses and fruit (Ciochon, Piperno & Thompson, 1990). In an extensive study on Hawaii, which included phytolith analysis, it was possible to identify when the native vegetation was altered and agricultural activities began (Pearsall & Trimble, 1984).

Pohl (1987) stated "Human evolution was influenced by the development of grasslands, and the development of great agricultural civilizations was made possible by graminaceous crops, including sugarcane, sorghum and maize". The evolution and early cultivation of these crops, and maize in particular, has therefore attracted a great deal of

attention, and phytolith analysis has been directed towards this investigation.

In 1977, Pearsall reported that maize (*Zea mays* subs. *mays*) could be identified from "extra-large cross" phytoliths when compared with known wild grasses, and used this to identify maize phytoliths from soil samples from Ecuador. The data from the phytolith analysis supported the argument for the cultivation of maize at 2450 B.C. in coastal Ecuador. Further analysis using both size and 3-dimensional form of phytoliths permitted discrimination between maize and wild grasses including teosinte (progenitor of maize), and allowed the presence of maize in Central Panama to be dated back to 5<sup>th</sup> millennium B.C. (Piperno, 1984). By using this improved classification method, Pearsall & Piperno (1990) argued that maize was present in coastal Ecuador 5000 - 6000 B.C. The use of phytoliths in maize identification was reviewed by Piperno (1988). Doolittle & Frederick (1991), however, found only a few "alleged diagnostic phytoliths" within the maize that they had grown, thus questioning the use of maize phytolith in such studies.

Investigations into crops, other than maize, using phytoliths are more limited, although Rosen (1989) tentatively suggested cereals were present in archaeological sites in the Galilee, Israel. Rosen (1992) further used silica skeletons (a number of phytoliths which remain cemented together as a replica of the original plant tissue) to discriminate between *Triticum*, *Hordeum*, *Phoenix dactylifera* (date palm), *Aegilops*, *Avena* and *Lolium*. One of the types of phytoliths measured was the papilla (plural, papillae).

### The Papilla.

Papillae have been observed and classified under various names. Prat (1933) described them as "cellules à pointe courte" and designated them as Po. The phytolith in Festucoid Class (Ia) 1a-circular in the report by Twiss *et al.* (1969) appears to be a papilla, as do the "conical-shaped phytoliths", and the possibly "hat-shaped silica bodies" reported by Piperno (1985). In Mulholland & Rapp's (1985) classification papillae were placed in the trichome class. Kaplan *et al.* (1992) appeared to classify papillae



twice - once as a sub division of "long cell appendages" termed "small prickles (IIa)" and also as a "trichrome base IIIb". The name papilla will be used throughout this study.

An interesting aspect of this particular phytolith was demonstrated by two reports. The first was by Hayward & Parry (1980), who reported that papillae from the inflorescence bracts of barley (*Hordeum vulgare*) possessed 7 - 9 depressions (pits) in the underside of its rim. They suspected that these depressions were points of contact with the underlying phytolith. The second study was an analysis of silica deposits in inflorescence bracts of *Triticum aestivum* cv. Highbury (Hodson & Sangster, 1988). It was noted that papillae from this species had 10 - 12 depressions (pits), and this led the authors to suggest that pit number had possible taxonomic significance.

This section of thesis aims to analyze one phytolith, the papilla, in numerous species/accessions. This is in contrast to previous studies (see above) which have attempted to analyse all the phytoliths produced by a particular plant. The investigation aims to determine the possible taxonomic significance of the papilla by analyzing the parameters of diameter and pit number from a large number of species/cultivars of both barley and wheat and also to include *Aegilops*, *Secale cereale* (rye) and *x Triticosecale* (triticale cultivars).

## 2.2. Materials and Methods.

### 2.2.1. Plant material.

Mature inflorescence spikes from different species/cultivars of wheat, barley, *Aegilops*, *Secale cereale* and 2 cultivars of *x Triticosecale* (triticale) were obtained from the sources listed below [all wheat and *Aegilops* nomenclature is after Miller (1987) and *Hordeum* species follow Hubbard (1984)]:

*Triticum boeoticum* Boiss ssp. *aegilopoides* (this will be termed *T. boeoticum* in the present investigation), *T. carthlicum* Nevski, *T. dicoccoides* (Korn) Schweinf., *T. dicoccum* (Schrank.) Schubl., *T. durum* Desf. cv. Kubanka, *T. macha* Dek. & Men., *T. monococcum* L., *T. paleocolchicum* Men., *T. polonicum* L., *T. sinskajae* A. Filat & Kurk., *T. spelta* L., *T. spelta* L. var. grey Spelta, *T. sphaerococcum* Perc., *T. boeoticum* Boiss ssp. *thaoudar* (this will be termed *T. thaoudar* in the present investigation), *T. timopheevi* Zhuk., *T. turanicum* Jakubz., *T. turgidum* L. cv. Rampton Rivet, *T. urartu* Tum., *T. vavilovi* (Yum.) Jakubz. and *T. zhukovskyi* Men. and Er. were all obtained from the Cambridge Laboratory, John Innes Institute, Norwich, England.

Ten old cultivars of *Triticum aestivum* L. (Squareheads Master, Little Joss, Desprez 80, Browick, Red Fife, Yeoman, Highbury, Benoist 40, Vilmorin 27, Prof. Delos) together with five modern cultivars (Troy, 90/3, 90/4, 90/6, 1757/58) were obtained from the Plant Breeding Institute, Cambridge, England.

Seven modern Canadian cultivars of *T. aestivum* L. were a gift from the University of Guelph, Ontario Agricultural College, Canada. These comprised the red wheats, Absolvent, Karat and Perlo, and the white wheats, Fredrick, Houser, Augusta and Frankenmuth.

*Hordeum jubatum* L. was collected in the Don Valley, Toronto, Canada. *H. murinum* L. was collected from a roadside in Headington, Oxford, England. *H. secalinum* L. was collected from Port Meadow, Oxford, England. *H. vulgare* L. old cultivars (Kenia, Goldthorpe, Bere, Chevalier, Plumage) were obtained from the Plant Breeding Institute,

Cambridge, England as were the modern cultivars of *H.vulgare* (CHE 4204/96, FR 79/39/3, 1098/804).

*Aegilops searsii* Feld. and Kis., *Ae. speltoides* Tausch, *Ae. speltoides* var. *aucheri* Boiss, *Ae. squarrosa* L. and *Ae. umbellulata* Zhuk., were obtained from the Cambridge Laboratory, John Innes Institute, Norwich, England.

Also obtained from the Cambridge Laboratory, John Innes Institute, Norwich, England was *Secale cereale* L. cv. King II. Triticale cv. OAC-Decade and triticale cv. OAC-Trillium was acquired from University of Guelph, Ontario Agricultural College, Canada.

Further gifts from the Cambridge Laboratory, John Innes Institute, Norwich, England were specific hybrids and their parents. These were: *T. dicoccum* x *Ae. squarrosa*, *T. boeoticum* x *Ae. speltoides* and *Ae. speltoides* x *T. monococcum*.

Grain from *T. boeoticum* were grown in pots containing a peat-based compost during the summer of 1993 in Oxfordshire, and the bracts produced from these plants, termed *T. boeoticum* (2), were also analyzed.

### 2.2.2. Microscopical Methods.

The glumes and lemmas were removed separately (using a stereo microscope where necessary) from the spikelets (except *Hordeum jubatum* where the glumes were reduced to awns). Tweezers were used to pull off the bracts from the spikelet except when the spikelet was very tough when the bracts had to be cut off using a scalpel. Any awns on the bracts were removed.

The glumes and lemmas were collected separately in nickel crucibles (25 ml capacity) until there was sufficient plant material to fill the crucible about three-quarters full. The sample was then cut into small pieces, about 5 mm x 5 mm squares. From this a small sample was taken. This sample was placed between two glass microscope slides, and then this and the sample in the nickel crucible were ashed in a Gallenkamp muffle furnace

at 420°C for 18 hours.

For light microscopy, the glass slides were carefully separated and a drop of Canada balsam (Sigma Chemicals, Poole, Dorset) was added and covered with a coverslip. This was then dried over a gentle heat on a hot plate overnight. This preparation was viewed with a Vickers MSC light microscope. For both glume and lemma, the diameters of 50 papillae were measured and the number of pits in the base of 50 papillae was recorded. Samples were photographed using a Zeiss standard microscope (with green illumination) with a Zeiss M35 camera fitted using Ilford HP5 black and white film.

For scanning electron microscopy, the ash from the crucible was mounted, using tweezers, on aluminium Cambridge stubs using double sided tape and sputter-coated with gold (180 sec.) using a Bio Rad sputter coating unit (Series II HD). The ash was viewed and photographed (the film was Kodak Technical Pan T110) using a JEOL T220 SEM, using secondary electron image detection. The operating voltage was 15 or 20 kV and the operating distance was 10-15 mm. The number of pits in the base of 50 papillae from both glume and lemma was recorded.

## 2.3. Results

### 2.3.1. Microscopical Studies.

Light micrographs of ashed inflorescence bracts are presented in figure 2.1. The diameter of the papillae are easily determined at the light microscope level (see fig. 2.1a), although, pit number is more difficult (fig 2.1b and 2.1c). All the pits of the papilla were rarely in the same plane of focus, and therefore to count pit number it was necessary to adjust the focus continually. By comparing papillae from *Ae. squarrosa* (fig. 2.1a) and *T. aestivum* cv. Absolvent (fig. 2.1b) to the papillae produced by *H. jubatum* (fig. 2.1c), which are all at the same magnification, it can clearly be seen that, typically, *Aegilops* sp. and *Triticum* sp. have larger papillae than *Hordeum* sp..

Figure 2.1c and 2.1d show other silicified cells which are present in the ashed inflorescence samples. *H. jubatum* (fig. 2.1c) shows small crescent-shaped silica cells, which are intercellular silica deposits and the sample from *T. durum* displays the silicified guard cells of stomata. The finer details of the structure of the papillae and other phytoliths are more clearly seen using the SEM.

Representative micrographs of the *Aegilops* sp., in this study, are presented in figure 2.2. Large areas of the inflorescence bract remain "cemented" together after the ashing procedure (fig. 2.2a), thus allowing papillae to be viewed *in situ*. At higher magnifications (fig. 2.2b) the papillae can clearly be observed as small conical projections on the surface of the bract. Greater detail of the underlying silicified dendriform cells can also be seen. Micrographs of the underside of the papillae are presented in figures 2.2c-f, and it is possible to see the pits within the rim of the papilla. Similarly to the light micrographs, it is not always possible to see all the pits in a single papilla without tilting the sample in the SEM.

Figure 2.3a show the underside of a papilla, from *Ae. speltoides* lemma, embedded in the long cells. On the right side of the papilla it appears that "projections" from the long cell "fit into" pits to the side of the papilla. This is more easily seen at higher magnification in figure 2.3b.

**Figure 2.1.** Light micrographs of papillae present in the ashed inflorescence bracts from *Aegilops*, *Triticum* and *Hordeum* species.

Examples of papilla are indicated by 'p' (x 600).

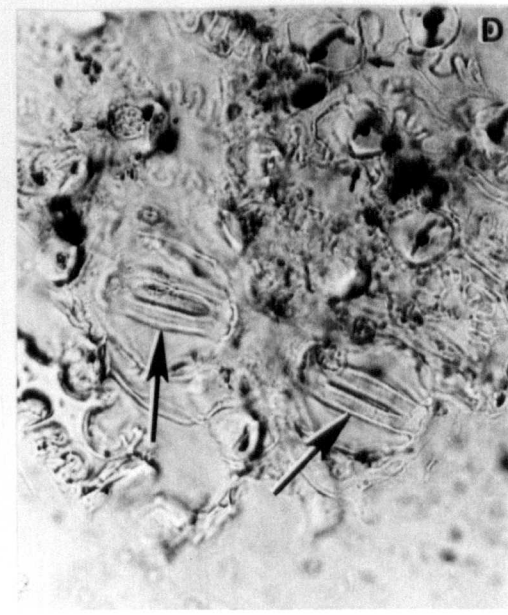
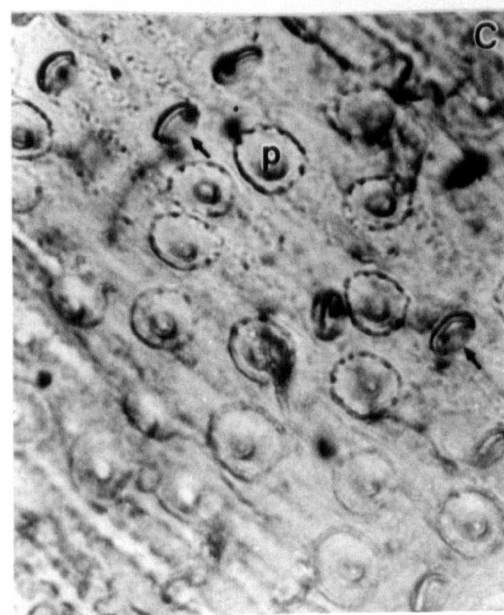
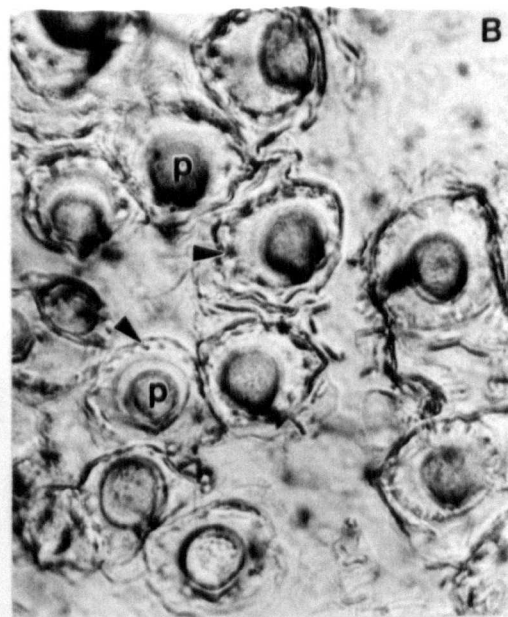
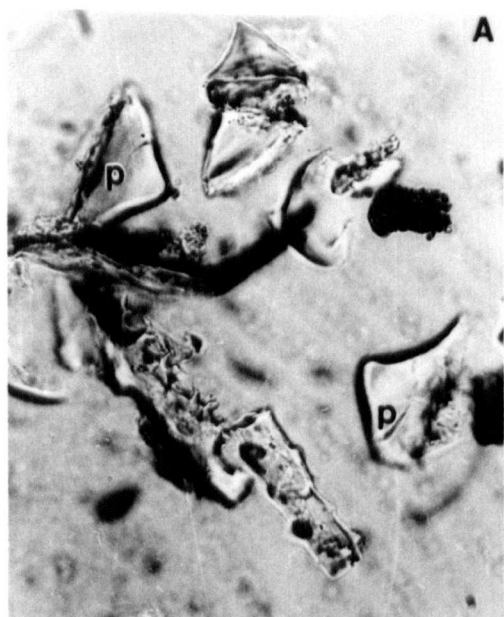
A) Papillae can clearly be seen in this sample from *Ae. squarrosa* lemma.

The diameter of the papilla is measured across the base.

B) The pits around the rim of the papilla can be seen (arrow). This sample is from *Triticum aestivum* cv. Absolvent lemma.

C) As well as papillae embedded in long cells, small silica cells are present (arrow) in this *H. jubatum* lemma sample.

D) Guard cells (large arrow) and silicified cork cells (small arrow) are clearly visible in *T. durum* cv. Kubanka glume.



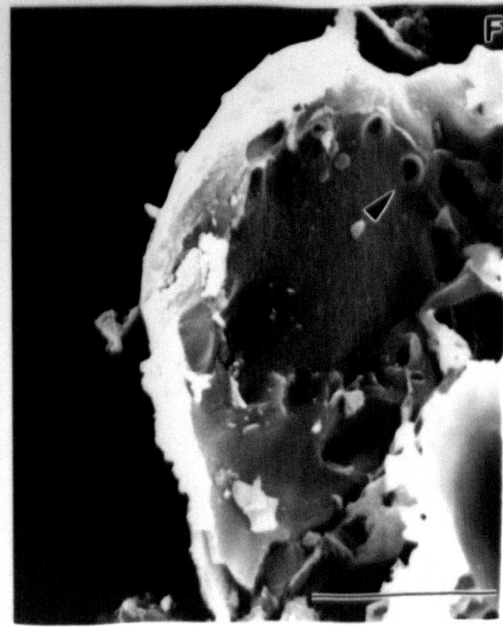
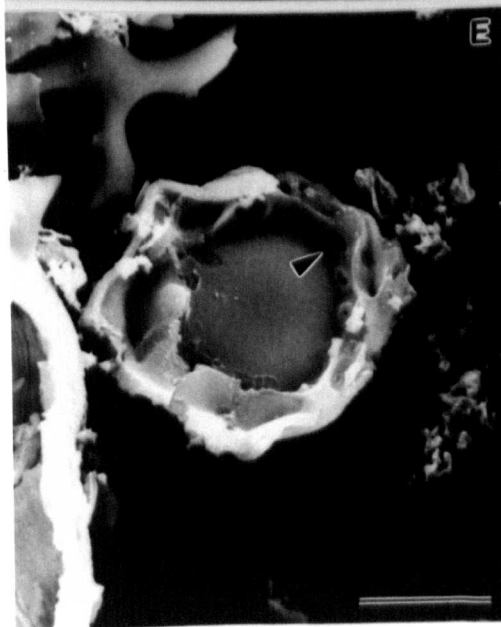
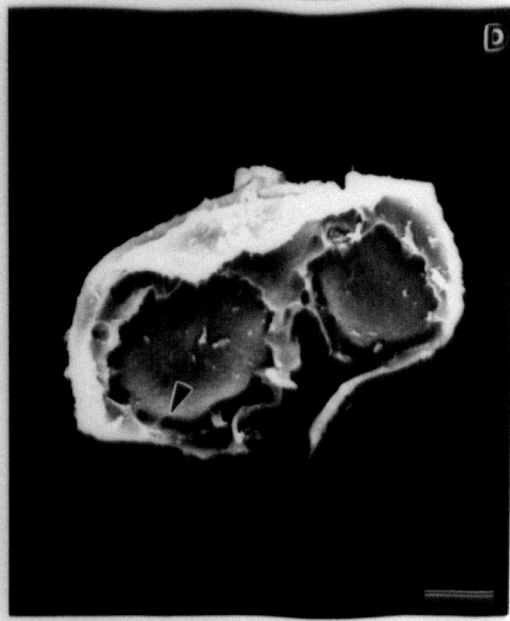
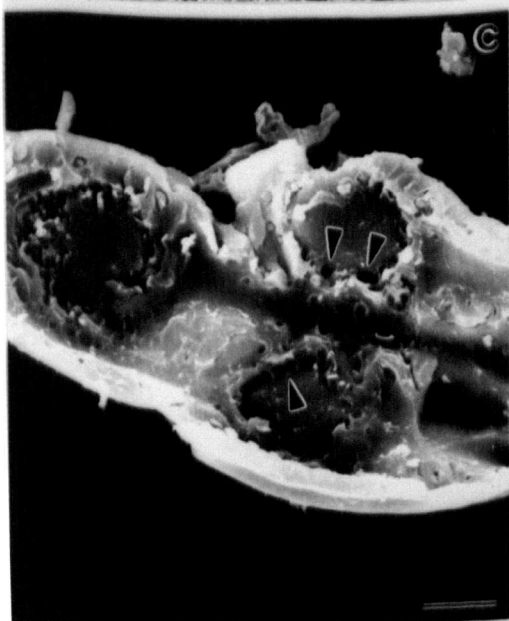
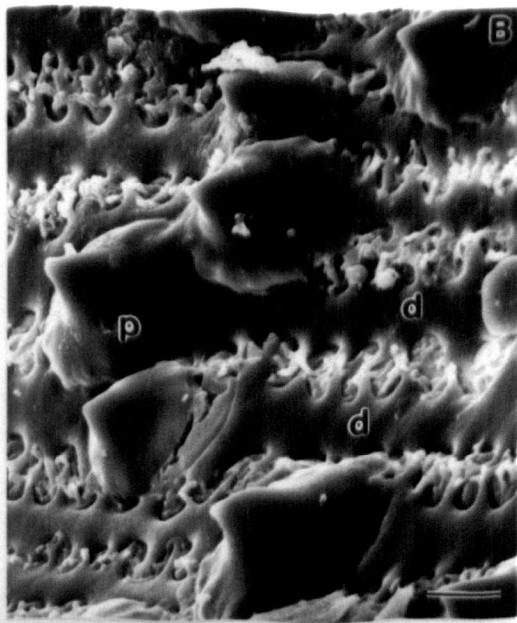
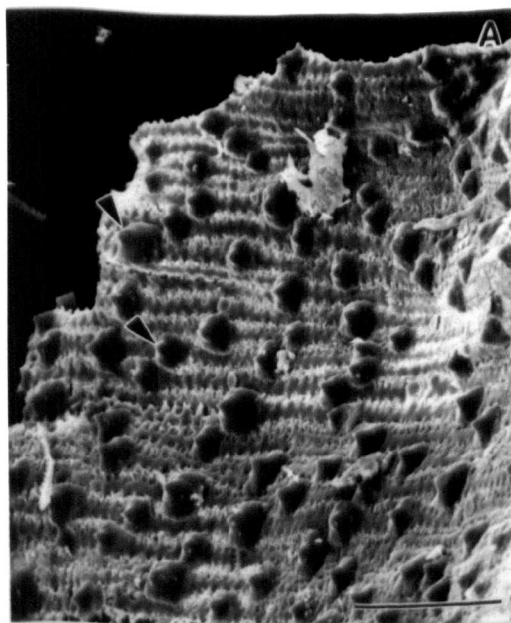
**Figure 2.2.** Scanning Electron Micrographs of papillae present in ashed inflorescence samples from *Aegilops* species.

A) Papillae can be seen on the surface of the inflorescence lemma from *Ae. squarrosa* (arrows indicate examples of papillae). Bar=100µm.

B) Figure 1a at higher magnification. The fine interlocking nature of the dendriform cells can be seen (p=papilla, d=dendriform). Bar=10µm.

C-F) Examples of pits in the underside of the papillae rim can be seen (arrows). Bar=10µm. C) *Ae. squarrosa* lemma. D) *Ae. speltoides* var. *aucheri* glume. E) *Ae. searsii* glume. F) *Ae. speltoides* lemma.





**Figure 2.3.** Scanning electron micrographs of papillae present in ashed inflorescence samples from *Aegilops* and *Hordeum* species.

A-B) *Ae. speltiodes* lemma. Projections (arrow) from the neighbouring dendriform cell can be seen to "fit in to" pits to the side of the papilla base.

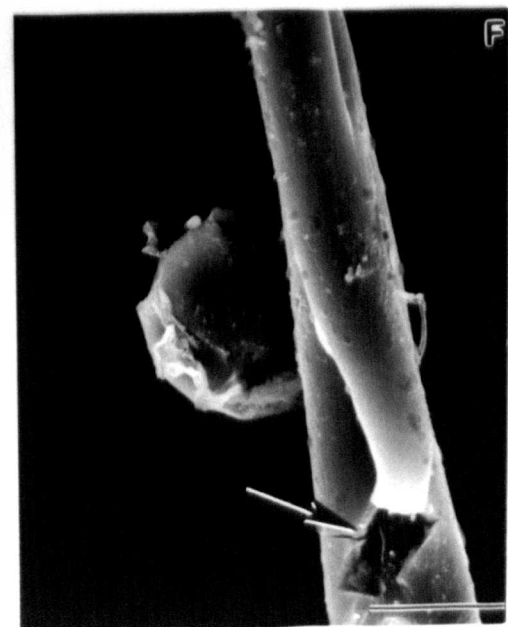
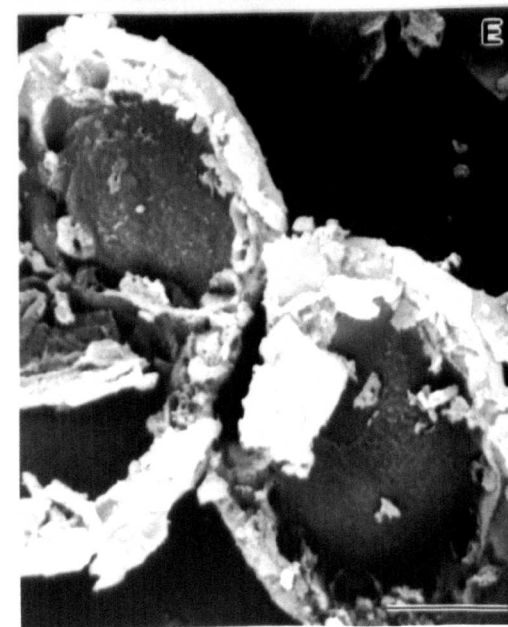
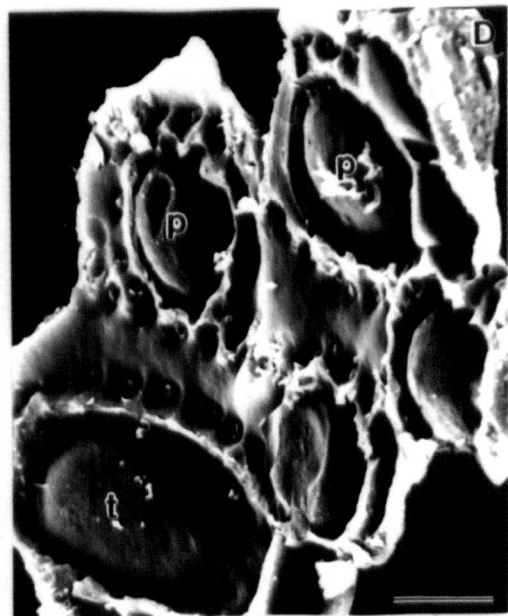
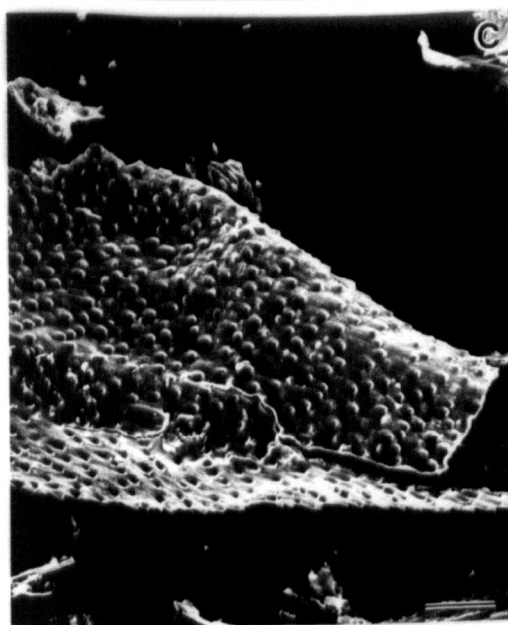
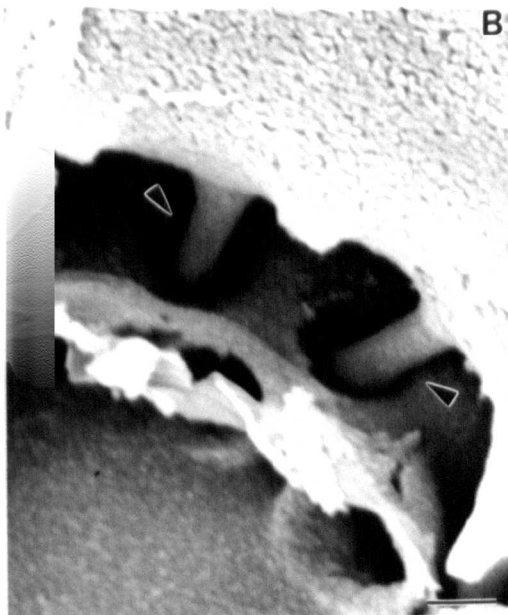
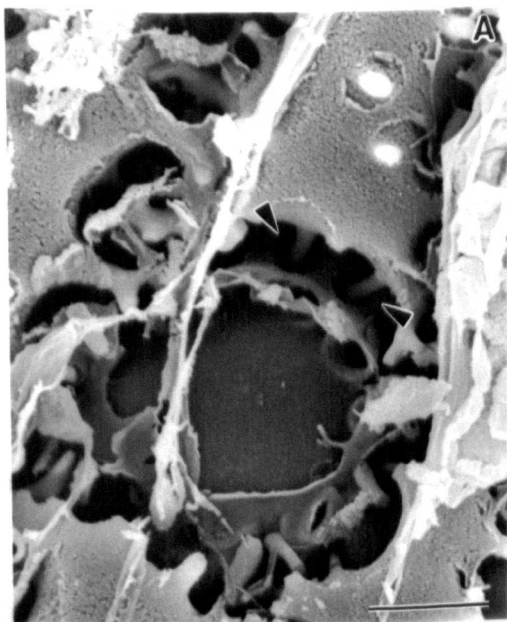
A) Bar=5 $\mu$ m. B) Bar=1 $\mu$ m.

C) Papillae on the outer surface of the lemma from *Hordeum vulgare* cv. Goldthorpe. Bar=100 $\mu$ m.

D) *Hordeum vulgare* cv. CHE 4204/96, lemma. The structure on the right is a trichome base (t). This contrasts with the underside of papillae (p) in both size and form. It can also be seen that the rim of the papillae obscures the pits slightly. Bar=10 $\mu$ m

E) The pits are more clearly seen in this sample from *H. jubatum* lemma. Bar=5 $\mu$ m.

F) The pits in this papilla are difficult to see. The hair appears to be hollow (arrow), *H. murinum*. Bar=10 $\mu$ m.



An extensive area of lemma from *H. vulgare* cv. Goldthorpe is presented in figure 2.3c, and large numbers of papillae can be seen covering the outside surface. Figure 2.3d (from *H. vulgare* cv. CHE 4204/96) shows the rim of the papillae tends to curve over the pits and obscure them slightly from view. Macrohair base are larger and more oval in shape than papillae bases, as can be seen in figure 2.3d. In *H. jubatum* (fig. 2.3e) it is possible to see pits within the rim of papillae. The hair in figure 2.3f (*H. murinum*) appears to be hollow.

Representative papillae from the *Triticum* sp. in this present investigation, are shown in figures 2.4, 2.5a and 2.5b. It can clearly be seen from figure 2.4a (*T. boeoticum*, lemma) that the tip of the papilla is not central over the base. It also appears that "projections" from the underlying cell "fit in to" the papilla base (indicated), this is similar to those seen in figure 2.3a and b. Figures 2.4b-f and 2.5a-b show the underside of papillae from various species of *Triticum*, displaying pits within the rim. Typically, *Triticum* sp. papillae, and those from *Aegilops* sp., did not show the curved rim seen in *Hordeum* sp. (compare figs. 2.4c and 2.2f with 2.3d). Papillae from *T. aestivum* (cv. Absolvent, fig. 2.4f) show a greater number of pits compared to species such as *T. boeoticum* (fig. 2.4c) and *T. carthlicum* (fig. 2.4e).

The papillae from the offspring of specific crosses are presented (fig. 2.5c-f). The variation in papilla diameter can be seen in figure 2.5c from *T. dicoccum* x *Ae. squarrosa*. This micrograph also demonstrates the papilla tips are off-centre and tend to point in the same direction. The crosses were between *Aegilops* sp. and *Triticum* sp. and the papillae appear similar to those produced by *Aegilops* sp. (see fig. 2.2) and *Triticum* sp. (see fig. 2.4).

### 2.3.2. Numerical Data.

The papillae data, for pit number and diameter, for glume, lemma and overall is presented in tables 2.2-2.5, for every species studied in the present investigation. This provides a basis for a database on papillae parameters, but is not necessarily the best way to present the data in a clear

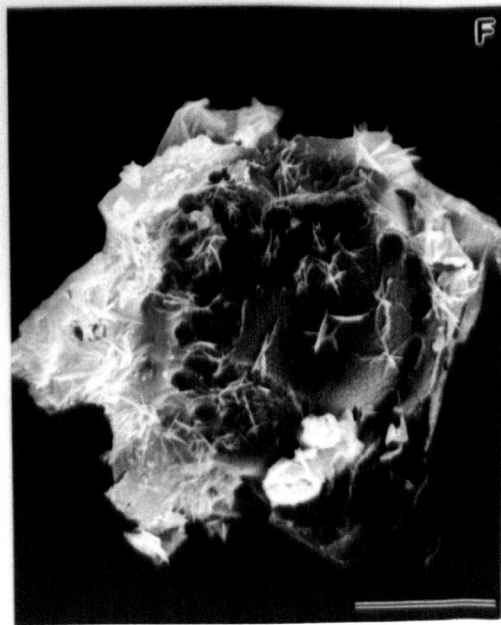
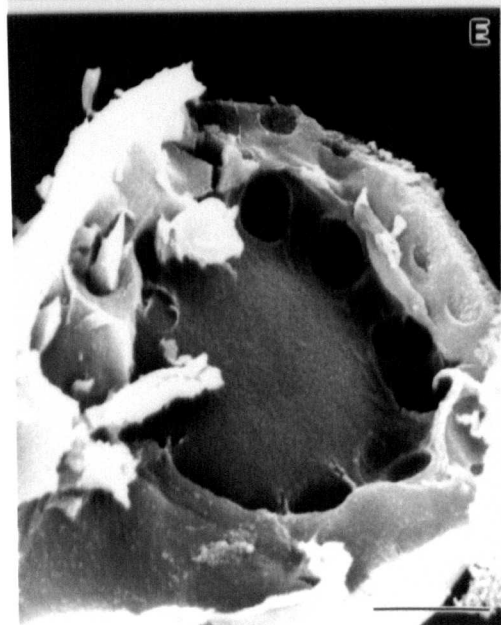
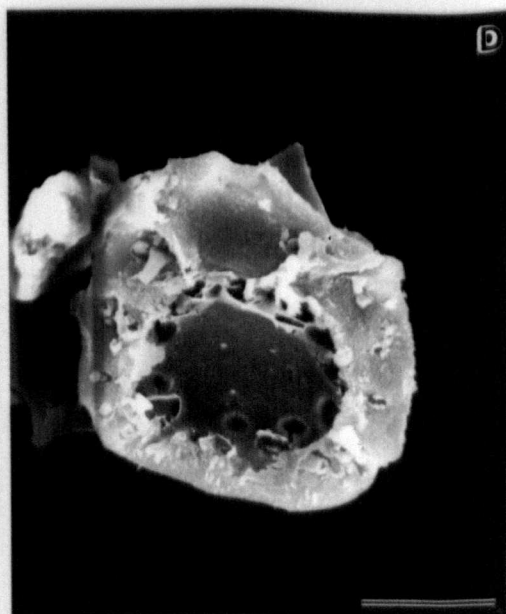
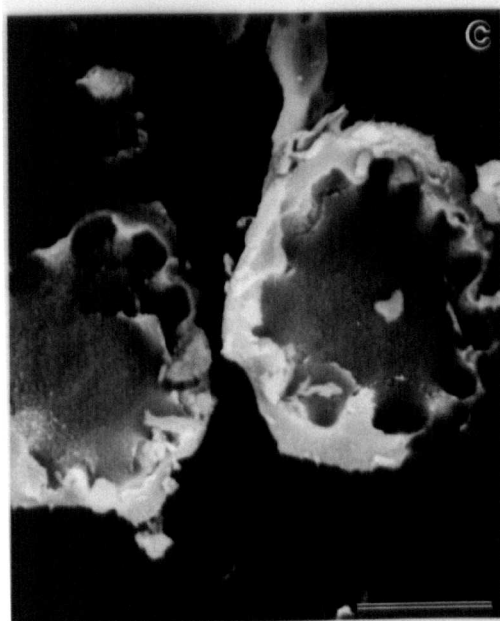
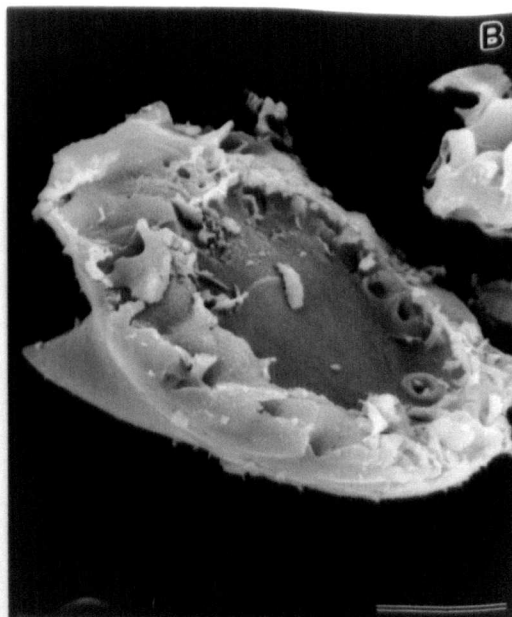
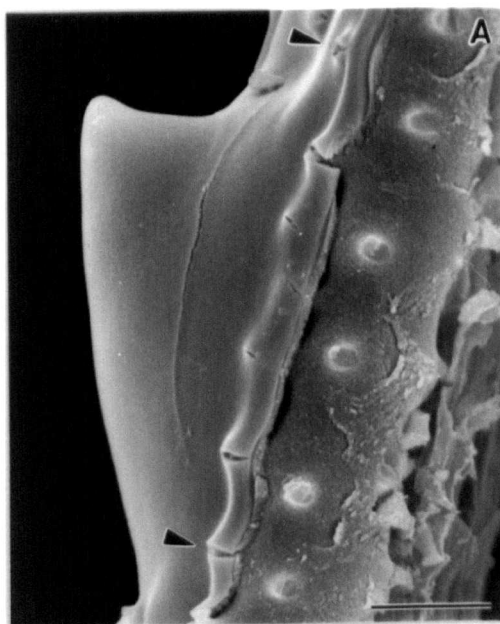
**Figure 2.4.** Scanning electron micrographs of papillae from the inflorescence bracts of *Triticum* species.

A) Projections from the underlying cell appear to fit in the rim of the overlying papilla (arrow), in this sample from *T. boeoticum* lemma.

Bar=5µm.

B-F) The pits in the rim of the papilla can clearly be seen in all these micrographs. B) Lemma from *T. paleocolchicum*. Bar=10µm C) Lemma from *T. boeoticum*. Bar=10µm D) Lemma from *T. zhukovskyi*.

Bar=10µm. E) Glume from *T. carthlicum*. Bar=5µm. F) Glume from *T. aestivum* cv. Absolvent. Bar=10µm.



**Figure 2.5.** Scanning electron micrographs of papillae from the inflorescence bracts of *Triticum* species and *Triticum* x *Aegilops* hybrids.

A-B) The underside of papillae from *Triticum* species showing the pits in the rim.

A) Glume from *T. vavilovi*. Bar=10µm.

B) Glume from *T. monococcum*. Bar=5µm

C-F) Papillae from the bracts of known crosses.

C) The outer area of the lemma of *T. dicoccum* x *Ae. squarrosa* hybrid, showing the top surface of papillae. Bar=10µm.

D) The underside of a papilla from the lemma of *T. dicoccum* x *Ae. squarrosa*, pits can clearly be seen in the rim. Bar=10µm.

E) Pits can be observed in the rim of this papilla from the glume of *Ae. speltooides* x *T. monococcum*. Bar=5µm.

F) In this cross, *Ae. speltooides* x *T. boeoticum*, the pits are more obscured by the rim of the papilla. Bar=10µm.

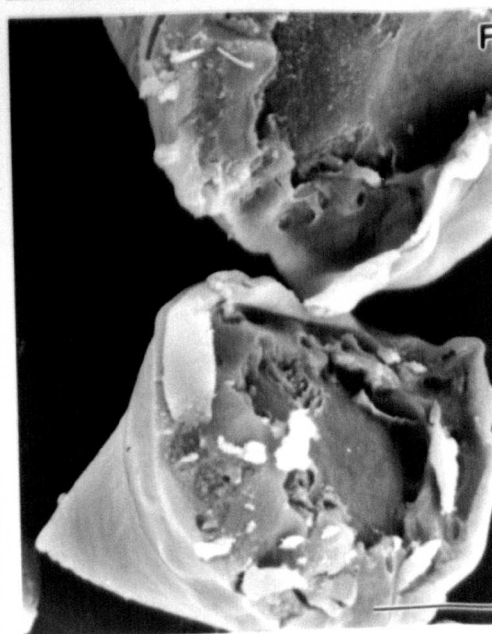
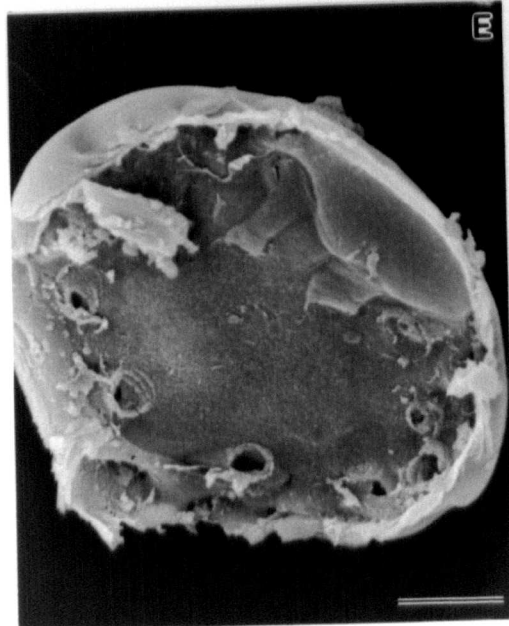
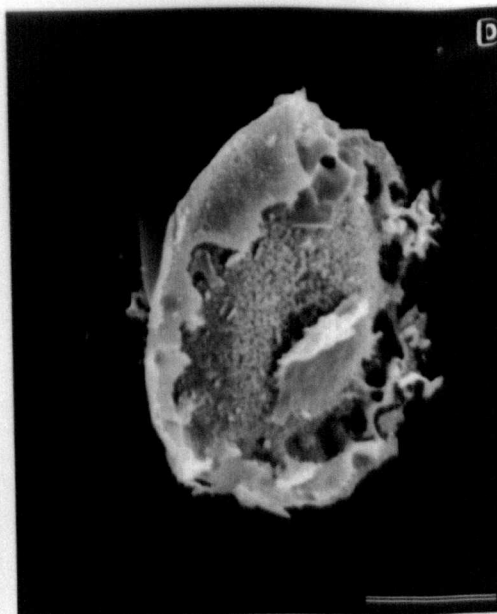
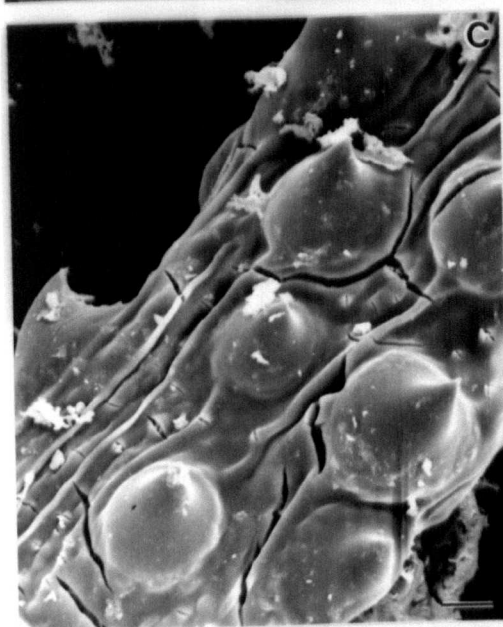
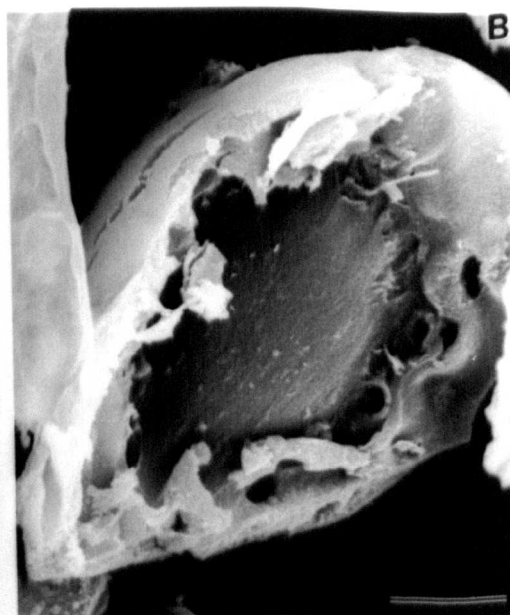
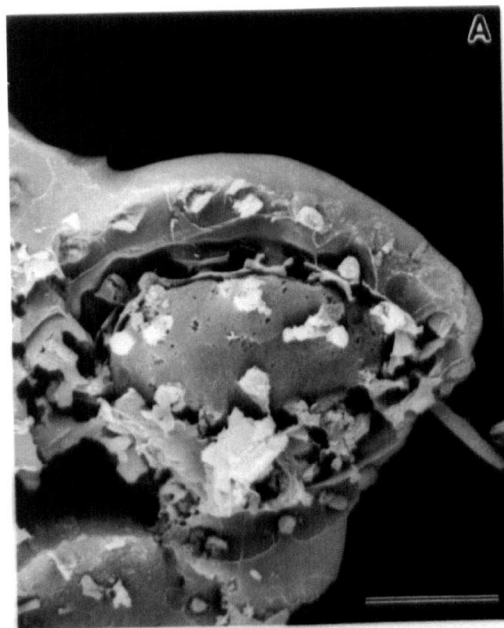




Table 2.2. Mean pit number and papilla diameter of the glume, lemma and overall data for *Hordeum* species.

Species	Cultivar	Glume		Lemma		Overall	
		Pit number (n=100)	Diameter (n=50)	Pit number (n=100)	Diameter (n=50)	Pit number (n=200)	Diameter (n=100)
<i>H. secalinum</i>		9.27 ± 1.3	18.83 ± 4.0	9.91 ± 1.4	17.94 ± 3.0	9.60 ± 1.4	18.40 ± 3.9
<i>H. jubatum</i>		*	*	11.41 ± 1.1	13.28 ± 1.7	11.40 ± 1.1*	13.30 ± 1.7*
<i>H. murinum</i>		9.37 ± 1.0	16.39 ± 2.7	8.41 ± 1.0	15.56 ± 2.2	8.90 ± 1.1	16.00 ± 2.5
<i>H. vulgare</i>	cv. Kenia	8.68 ± 0.9	13.97 ± 2.4	8.30 ± 1.0	14.32 ± 1.7	8.50 ± 1.0	14.10 ± 2.1
<i>H. vulgare</i>	cv. Goldthorpe	8.10 ± 1.0	14.13 ± 2.3	8.25 ± 1.0	15.72 ± 2.5	8.20 ± 1.0	14.90 ± 2.5
<i>H. vulgare</i>	cv. Bere	7.39 ± 0.8	13.50 ± 2.2	7.83 ± 1.0	14.69 ± 2.2	7.60 ± 0.9	14.10 ± 2.3
<i>H. vulgare</i>	cv. Chevalier	8.22 ± 1.0	14.25 ± 2.0	8.62 ± 1.0	15.25 ± 2.2	8.40 ± 1.0	14.80 ± 2.1
<i>H. vulgare</i>	cv. Plumage	7.51 ± 0.8	13.00 ± 1.5	7.89 ± 0.8	15.11 ± 1.9	7.70 ± 0.8	14.10 ± 2.0
<i>H. vulgare</i>	cv. CHE 4204/96	8.34 ± 0.8	15.26 ± 2.6	8.38 ± 0.9	16.57 ± 2.9	8.40 ± 0.8	15.90 ± 2.8
<i>H. vulgare</i>	cv. FR 79/39/3	8.14 ± 0.8	16.45 ± 1.9	8.03 ± 0.8	17.56 ± 2.7	8.10 ± 0.8	17.00 ± 2.4
<i>H. vulgare</i>	cv. 1098/804	8.25 ± 0.8	14.11 ± 2.2	8.36 ± 0.7	15.84 ± 1.9	8.30 ± 0.7	15.00 ± 2.2

Overall data represent the glume and lemma combined. All data are means ± standard deviations. \* In this species the glume is reduced to an awn and was not considered, therefore overall pit number n=100 and overall diameter n=50.

**Table 2.3.** Mean pit number and papilla diameter of the glume, lemma and overall data for *Aegilops* species.

Species	Glume		Lemma		Overall	
	Pit number (n=100)	Diameter (n=50)	Pit number (n=100)	Diameter (n=50)	Pit number (n=200)	Diameter (n=100)
<i>Ae. umbellulata</i>	11.47 ± 1.2	18.88 ± 3.0	10.69 ± 1.1	18.57 ± 2.9	11.10 ± 1.2	18.70 ± 2.9
<i>Ae. searsii</i>	11.93 ± 1.3	21.16 ± 3.1	12.10 ± 1.5	21.48 ± 2.9	12.00 ± 1.4	21.30 ± 2.8
<i>Ae. speltoides</i>	12.16 ± 1.4	21.38 ± 3.5	12.49 ± 1.7	21.06 ± 2.7	12.30 ± 1.3	21.20 ± 3.2
<i>Ae. speltoides</i> var <i>aucheri</i>	12.93 ± 1.6	27.83 ± 4.1	13.30 ± 1.4	26.91 ± 3.5	13.10 ± 1.5	27.40 ± 3.9
<i>Ae. squarrosa</i>	11.65 ± 1.4	22.75 ± 3.1	12.39 ± 1.5	21.75 ± 2.4	12.00 ± 1.5	22.30 ± 2.8

Overall data represent the glume and lemma combined. All data are means ± standard deviations.

**Table 2.4.** Mean pit number and papilla diameter of the glume, lemma and overall data for *Triticum* species, (other than *T. aestivum*) *Secale cereale* and Triticale.

Species	Genome Formula	Glume		Lemma		Overall	
		Pit number (n=100)	Diameter (n=50)	Pit number (n=100)	Diameter (n=50)	Pit number (n=200)	Diameter (n=100)
<i>T. boeoticum</i>	AA	8.36 ± 1.0	20.94 ± 2.6	8.50 ± 1.0	20.13 ± 2.7	8.40 ± 1.0	20.50 ± 2.7
<i>T. boeoticum</i> 2	AA	8.75 ± 1.3	22.24 ± 4.0	8.10 ± 1.4	18.71 ± 3.0	8.40 ± 1.4	20.50 ± 3.9
<i>T. thaoudar</i>	AA	10.51 ± 1.3	24.60 ± 4.4	10.90 ± 1.2	23.33 ± 3.4	10.71 ± 1.3	23.97 ± 3.9
<i>T. urartu</i>	AA	11.21 ± 1.2	26.85 ± 3.9	11.49 ± 1.4	25.69 ± 3.3	11.35 ± 1.3	26.27 ± 3.6
<i>T. sinskajae</i>	AA	10.17 ± 1.3	23.13 ± 3.3	11.30 ± 1.2	24.25 ± 3.5	10.73 ± 1.4	23.69 ± 3.4
<i>T. monococcum</i>	AA	7.49 ± 0.9	12.80 ± 1.7	8.62 ± 1.2	17.17 ± 2.3	8.10 ± 1.2	15.00 ± 2.9
<i>T. dicoccoides</i>	AABB	8.88 ± 1.1	19.63 ± 3.4	9.28 ± 1.0	20.69 ± 3.3	9.10 ± 1.1	20.20 ± 3.4
<i>T. dicoccum</i>	AABB	7.93 ± 0.9	22.31 ± 3.3	9.81 ± 1.0	17.50 ± 2.8	8.90 ± 1.3	19.90 ± 3.9
<i>T. turgidum</i> cv. Rampton	AABB	8.35 ± 0.8	15.38 ± 2.7	8.67 ± 1.1	16.69 ± 2.2	8.50 ± 1.0	16.00 ± 2.5
<i>T. durum</i> cv. Kubanka	AABB	9.96 ± 1.2	18.38 ± 3.1	8.34 ± 0.8	26.07 ± 2.7	9.20 ± 1.3	22.30 ± 4.8
<i>T. carthlicum</i>	AABB	11.31 ± 1.2	22.06 ± 3.0	11.33 ± 1.2	23.27 ± 4.4	11.32 ± 1.2	23.38 ± 3.8
<i>T. paleocolchicum</i>	AABB	10.33 ± 1.3	16.97 ± 3.2	9.77 ± 1.3	22.57 ± 3.6	10.05 ± 1.3	19.77 ± 4.4

Overall data represent the glume and lemma combined. All data are means ± standard deviations.

Continued overleaf.

Table 2.4. continued.

Species	Genome Formula	Glume		Lemma		Overall	
		Pit number (n=100)	Diameter (n=50)	Pit number (n=100)	Diameter (n=50)	Pit number (n=200)	Diameter (n=100)
<i>T. turanicum</i>	AABB	10.00 ± 1.3	28.34 ± 5.0	10.06 ± 1.2	24.91 ± 4.3	10.03 ± 1.2	26.63 ± 5.0
<i>T. polonicum</i>	AABB	10.55 ± 1.3	28.28 ± 6.7	10.89 ± 1.4	26.36 ± 3.2	10.72 ± 1.3	27.32 ± 4.5
<i>T. sphaerococcum</i>	AABDDD	11.07 ± 2.0	30.12 ± 5.9	10.95 ± 1.2	28.36 ± 4.8	10.96 ± 1.7	29.24 ± 5.4
<i>T. macha</i>	AABDDD	11.98 ± 1.4	29.38 ± 4.4	11.42 ± 1.0	28.63 ± 4.0	11.69 ± 1.3	29.00 ± 1.3
<i>T. vavilovi</i>	AABDDD	10.51 ± 1.6	27.06 ± 2.9	10.90 ± 1.3	26.56 ± 4.5	10.71 ± 1.3	26.81 ± 3.8
<i>T. spelta, grey</i>	AABDDD	11.38 ± 1.1	27.12 ± 4.9	11.81 ± 1.5	22.12 ± 4.9	10.36 ± 1.6	29.24 ± 5.0
<i>T. spelta</i>	AABDDD	9.60 ± 1.2	30.30 ± 3.6	11.13 ± 1.2	28.18 ± 3.3	11.60 ± 1.2	24.60 ± 4.2
<i>T. timopheevi</i>	AAGG	8.34 ± 0.8	16.88 ± 2.3	9.09 ± 1.1	16.07 ± 1.9	8.70 ± 1.0	16.50 ± 2.1
<i>T. zhukovskiy</i>	AAAAGG	10.50 ± 1.2	17.13 ± 2.9	10.08 ± 1.0	22.17 ± 3.3	10.30 ± 1.1	19.70 ± 4.0
<i>Secale cereale</i>	RR	8.78 ± 1.2	16.97 ± 2.4	8.80 ± 1.1	22.81 ± 3.0	8.80 ± 1.1	19.90 ± 4.0
<i>Triticale</i> OAC-Decade	RRAABB	9.58 ± 1.1	14.63 ± 2.3	11.18 ± 1.6	27.37 ± 3.5	10.30 ± 1.6	21.00 ± 7.0
<i>Triticale</i> OAC-Trillium	RRAABB	9.06 ± 1.1	16.38 ± 3.2	9.54 ± 1.4	23.44 ± 3.8	8.95 ± 1.3	20.10 ± 5.0

Overall data represent the glume and lemma combined. All data are means ± standard deviations.

**Table 2.5.** Mean pit number and papilla diameter of the glume, lemma and overall data for *Triticum aestivum* cultivars.

Species	Cultivar	Genome Formula	Glume		Lemma		Overall	
			Pit number (n=100)	Diameter (n=50)	Pit number (n=100)	Diameter (n=50)	Pit number (n=200)	Diameter (n=100)
<i>T. aestivum</i>	cv. Squareheads	AABDD	10.38 ± 1.1	16.72 ± 2.8	9.19 ± 1.0	19.39 ± 2.5	10.30 ± 1.1	18.10 ± 3.0
<i>T. aestivum</i>	cv. Little Joss	AABDD	9.25 ± 1.0	22.31 ± 3.8	10.27 ± 1.1	22.94 ± 3.0	9.70 ± 1.3	22.60 ± 3.4
<i>T. aestivum</i>	cv. Desprez 80	AABDD	9.73 ± 1.1	18.50 ± 3.1	10.23 ± 1.0	18.44 ± 2.3	9.80 ± 1.1	18.50 ± 2.7
<i>T. aestivum</i>	cv. Browick	AABDD	10.09 ± 1.0	23.37 ± 3.3	9.91 ± 0.9	23.22 ± 3.1	9.80 ± 1.0	23.30 ± 3.1
<i>T. aestivum</i>	cv. Red fife	AABDD	10.16 ± 1.0	23.83 ± 3.1	9.44 ± 1.0	23.28 ± 2.9	10.00 ± 1.0	23.60 ± 3.0
<i>T. aestivum</i>	cv. Yeoman	AABDD	9.31 ± 1.1	23.22 ± 3.2	9.77 ± 0.9	22.94 ± 3.0	9.40 ± 1.0	23.10 ± 3.1
<i>T. aestivum</i>	cv. Highbury	AABDD	11.06 ± 1.3	21.19 ± 3.1	9.47 ± 1.6	20.46 ± 2.8	12.50 ± 2.0	20.80 ± 3.0
<i>T. aestivum</i>	cv. Benoist 40	AABDD	10.04 ± 1.1	20.17 ± 3.0	13.90 ± 1.1	22.11 ± 3.3	10.00 ± 1.1	21.10 ± 3.3
<i>T. aestivum</i>	cv. Vilmorin 27	AABDD	9.92 ± 1.1	23.78 ± 2.8	10.00 ± 1.1	23.09 ± 3.3	10.20 ± 1.1	23.40 ± 3.1
<i>T. aestivum</i>	cv. Prof. Delos	AABDD	9.87 ± 1.0	23.37 ± 3.4	10.49 ± 1.0	25.26 ± 3.3	9.80 ± 1.0	24.30 ± 3.7
<i>T. aestivum</i>	cv. Troy	AABDD	10.85 ± 1.1	24.74 ± 3.1	9.70 ± 1.3	22.63 ± 2.8	11.10 ± 1.2	23.70 ± 3.1
<i>T. aestivum</i>	cv. 90/3	AABDD	11.16 ± 1.0	27.37 ± 3.9	11.44 ± 1.1	27.31 ± 2.8	11.00 ± 1.1	27.30 ± 3.3
<i>T. aestivum</i>	cv. 90/4	AABDD	10.72 ± 1.1	27.11 ± 2.9	10.74 ± 0.9	23.83 ± 2.7	10.70 ± 1.0	25.50 ± 3.3
<i>T. aestivum</i>	cv. 90/6	AABDD	12.50 ± 1.0	26.40 ± 2.6	10.65 ± 1.0	26.06 ± 3.8	12.40 ± 1.0	26.20 ± 3.2
<i>T. aestivum</i>	cv. 1757/68	AABDD	12.16 ± 1.2	24.91 ± 3.3	12.28 ± 1.2	26.80 ± 2.9	12.30 ± 1.2	25.90 ± 3.2
<i>T. aestivum</i>	cv. Absolvent	AABDD	14.05 ± 1.2	24.00 ± 4.4	14.57 ± 1.5	19.03 ± 3.1	14.30 ± 1.4	21.50 ± 4.6
<i>T. aestivum</i>	cv. Karat	AABDD	7.77 ± 0.9	14.03 ± 2.6	12.24 ± 1.2	19.19 ± 3.2	10.00 ± 2.5	16.60 ± 3.9
<i>T. aestivum</i>	cv. Perlo	AABDD	14.55 ± 1.3	19.16 ± 3.7	14.20 ± 1.2	22.03 ± 4.5	14.40 ± 1.3	20.60 ± 4.3
<i>T. aestivum</i>	cv. Fredrick	AABDD	14.84 ± 1.2	29.44 ± 5.3	11.95 ± 1.1	21.28 ± 3.0	13.40 ± 1.8	25.40 ± 6.0
<i>T. aestivum</i>	cv. Houser	AABDD	10.59 ± 1.1	19.72 ± 4.6	9.76 ± 1.3	23.56 ± 4.6	10.20 ± 1.2	21.60 ± 4.4
<i>T. aestivum</i>	cv. Augusta	AABDD	16.34 ± 1.3	28.13 ± 4.3	12.05 ± 1.0	27.63 ± 3.6	14.20 ± 1.2	27.90 ± 4.6
<i>T. aestivum</i>	cv. Frankenmuth	AABDD	11.57 ± 1.4	27.35 ± 4.5	10.29 ± 1.0	22.38 ± 3.2	10.90 ± 1.4	24.90 ± 4.6

Overall data represent the glume and lemma combined. All data are means ± standard deviations.

format. The data obtained using light and scanning electron microscopy was combined as both methods gave similar results, and, as glume and lemma did not show any consistent trends concerning papilla diameter or pit number, the overall data was used for the following analysis.

#### Comparisons within *Hordeum* species.

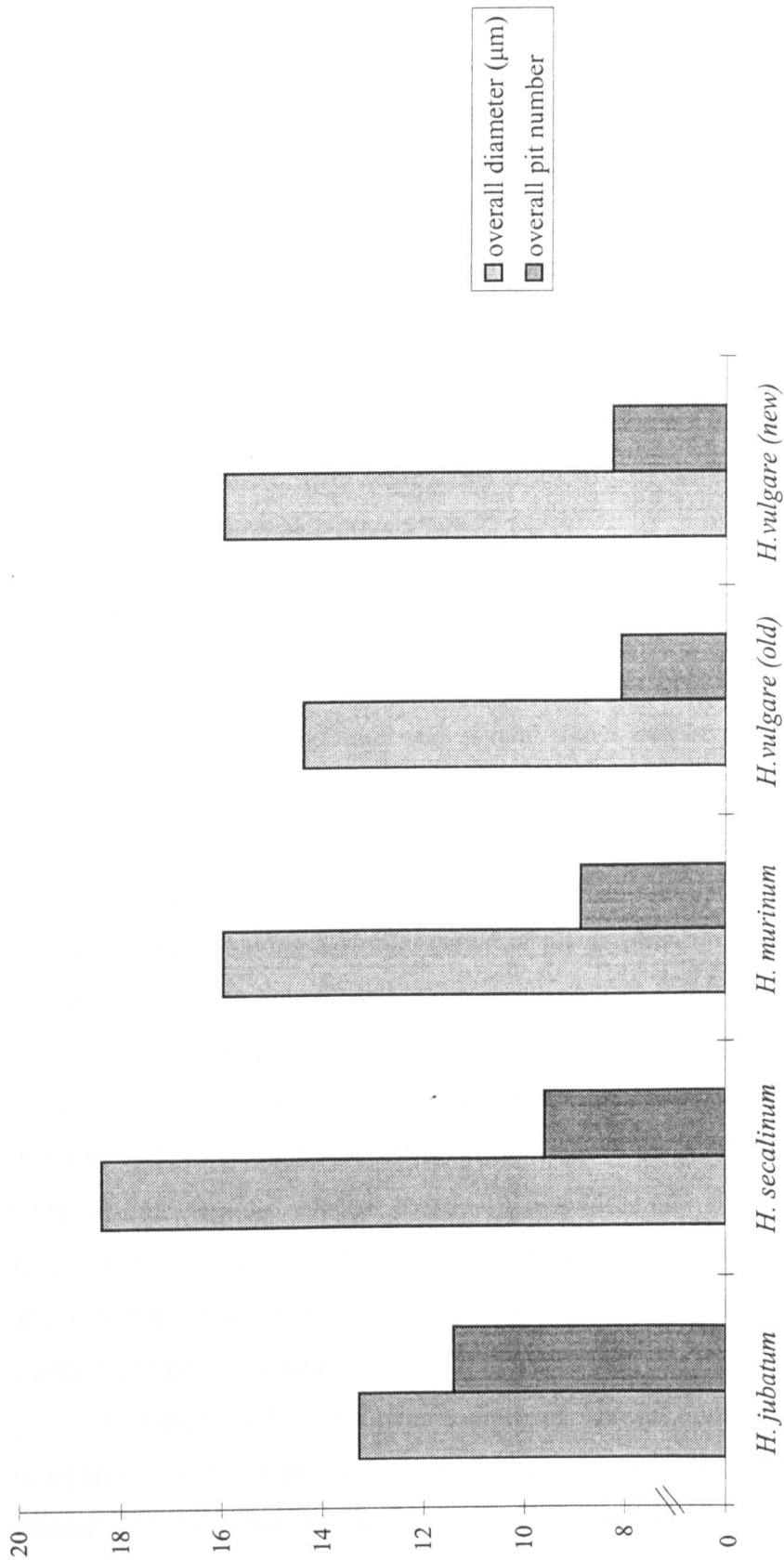
When comparing *Hordeum* species (tab. 2.2), *H. jubatum* has a significantly the highest pit number ( $p=0.05$ , d.f.= 298) and *H. murinum*, has significantly, the lowest number (except for *H. vulgare* cv. CHE 4204/96,  $p=0.05$ , d.f.=398), although *H. secalinum* has the largest papilla diameter of the barleys ( $p=0.05$ , d.f.=398) in the present study, see figure 2.6. This figure shows the mean average for all *H. vulgare* old cultivars [labelled *H. vulgare* (old)], and similarly for all *H. vulgare* new cultivars, labelled *H. vulgare* (new). The papillae parameters of *H. vulgare* cultivars tend to be more similar to *H. murinum* than to either *H. jubatum* or *H. secalinum*.

#### Comparisons within *Aegilops* species.

*Ae. speltoides* var. *aucheri* had significantly larger number of pits and larger papillae diameter (pit number: 13.10, diameter: 27.40  $\mu\text{m}$ ) than all the *Aegilops* species studied in the current investigation ( $p=0.05$ , d.f.=398). *Ae. umbellulata* had significantly smaller papillae parameters than the other *Aegilops* species (tab. 2.3).

#### Comparisons within Rye and Triticale.

One species of rye (*Secale cereale*), King II and two cultivars of triticale (synthetic hybrid between rye and *Triticum durum*) were analyzed and therefore, this data is very preliminary. The data in table 2.4 shows that rye has smaller papillae parameters than either triticale cultivar, although only pit number was significantly different ( $p=0.05$ , d.f.=398). Triticale cv. Decade had significantly larger number of pits overall than triticale cv. Trillium ( $p=0.05$ , d.f.=398).



**Figure 2.6.** A comparison of average overall pit number and papilla diameter (in  $\mu\text{m}$ ) between *Hordeum* species and *H. vulgare* cultivars. Old/new indicates age of formation of cultivar.

### Comparisons within *Triticum* species.

A large number of *Triticum* species was studied in the present investigation, including 22 different cultivars of *T. aestivum* (tab. 2.5). From figure 2.7, it can be seen that, as the genome of *Triticum* sp. increases from AA to AABB to AABBDD, the papillae diameter and pit number increases. In this figure (2.7) the results are mean averages of all the wheats possessing the appropriate genome designation for that group.

Canadian cultivars of *T. aestivum* have a higher number of papilla pits than British cultivars, although the latter has a larger papilla diameter (fig 2.8). It is also evident that the new British cultivars have more pits and greater papilla diameter than established (old) British cultivars. This is also the case with *H. vulgare* cultivars (fig. 2.6).

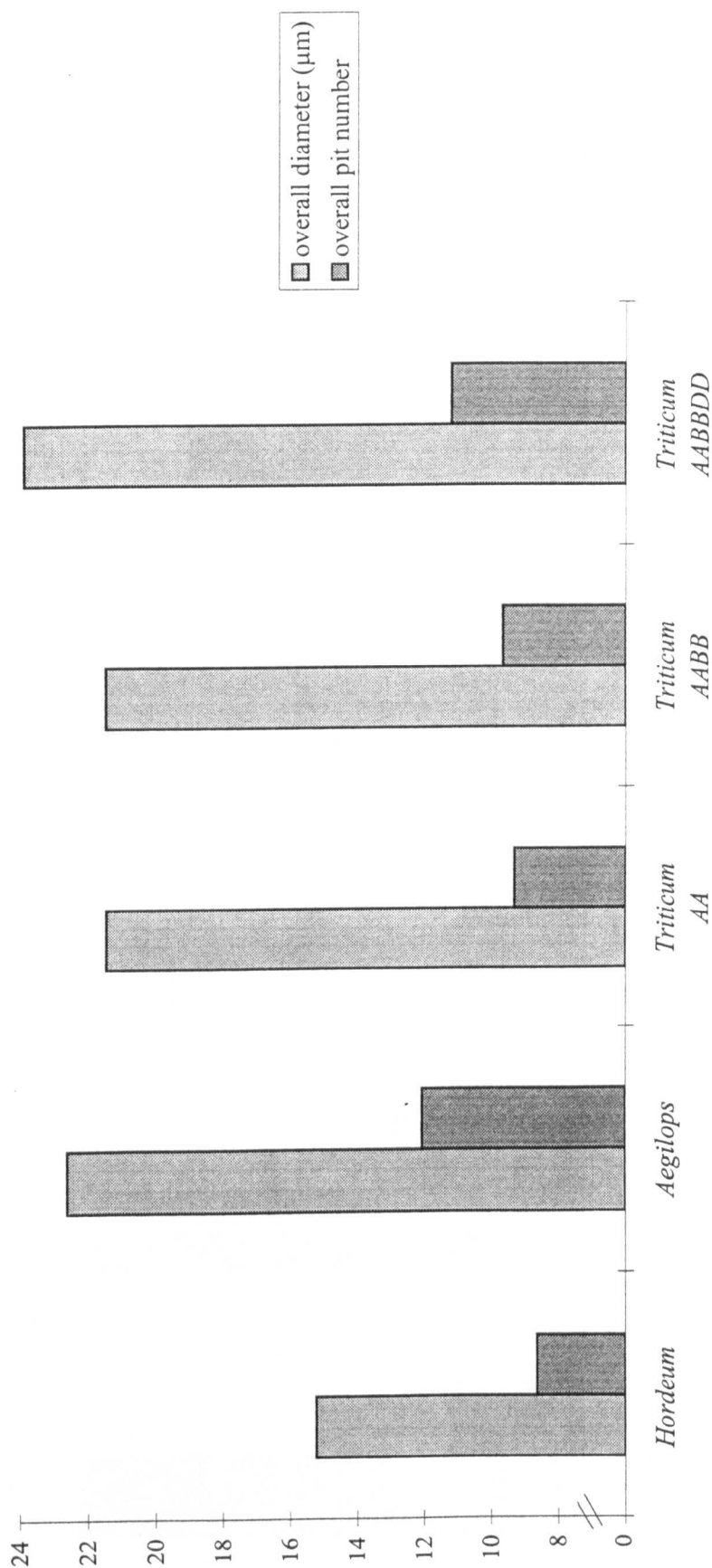
### General Comparisons.

A scattergram (fig. 2.9) shows the spread of species when grouped together by genome designation and several trends can be seen. *Hordeum* species tend to be at the lower range of both papillae parameters. *Triticum* species with the ABD genome (including *T. aestivum*, termed *Triticum* species AABBDD) tend to have the largest papilla diameter, while *Aegilops* species, although having a similar range of pit number, are inclined to possess smaller papilla.

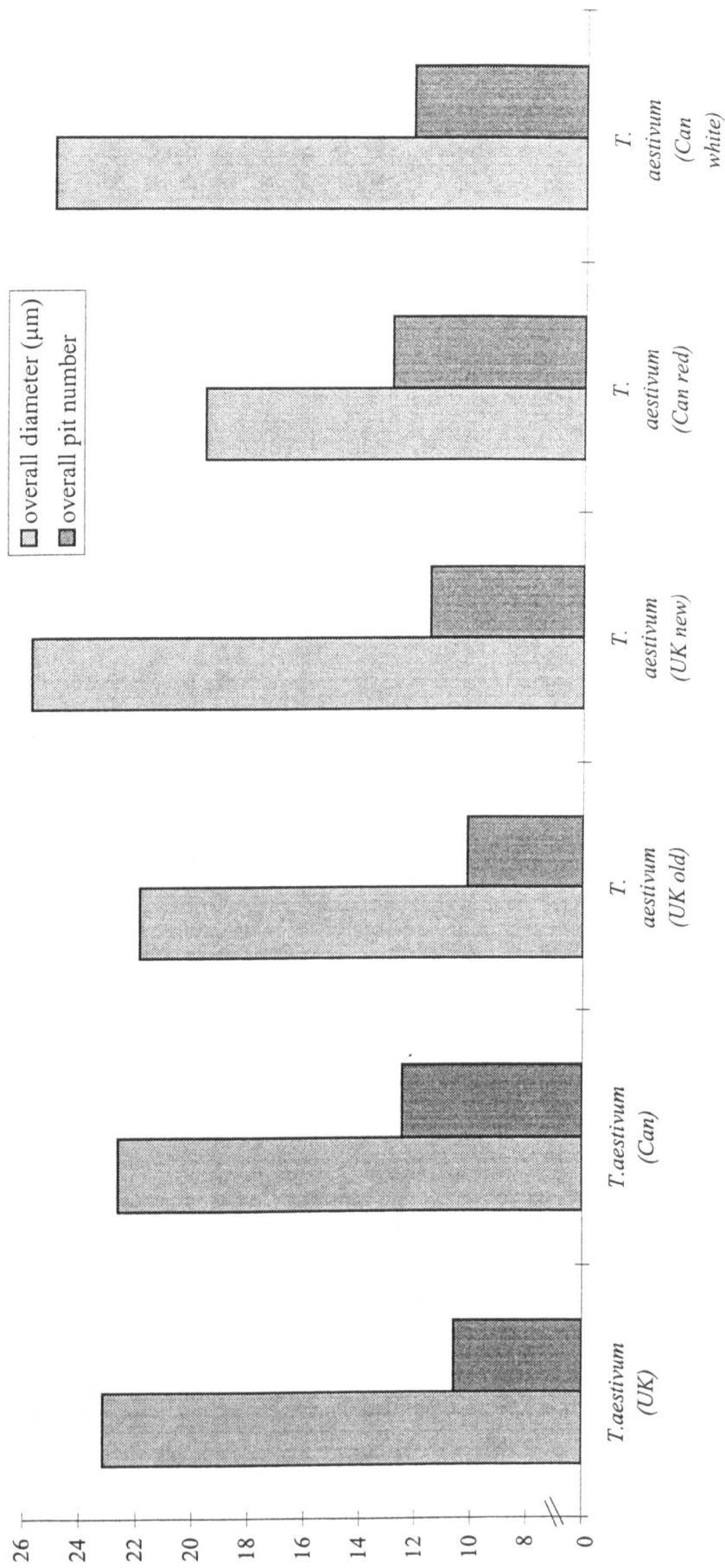
Analysis of correlations between pit number and papilla diameter showed there was very little correlation ( $r=0.6$ , d.f.=68) over the whole range of species in the present study. The highest correlation coefficients were for the *Aegilops* species group ( $r=0.8$ , d.f.=5) and *Triticum* species with genomic designation AA (termed *Triticum* species AA,  $r=0.8$ , d.f.=6) although these did not indicate a high degree of correlation between pit number and papilla diameter.

Comparisons between papilla diameter and pit number are presented in figures 2.6-2.8. Figure 2.7 shows that *Hordeum* species have lower pit number and smaller diameter than *Aegilops* sp. and *Triticum* sp.. The mean overall data for diameter and pit number for the groups *Hordeum* sp.,

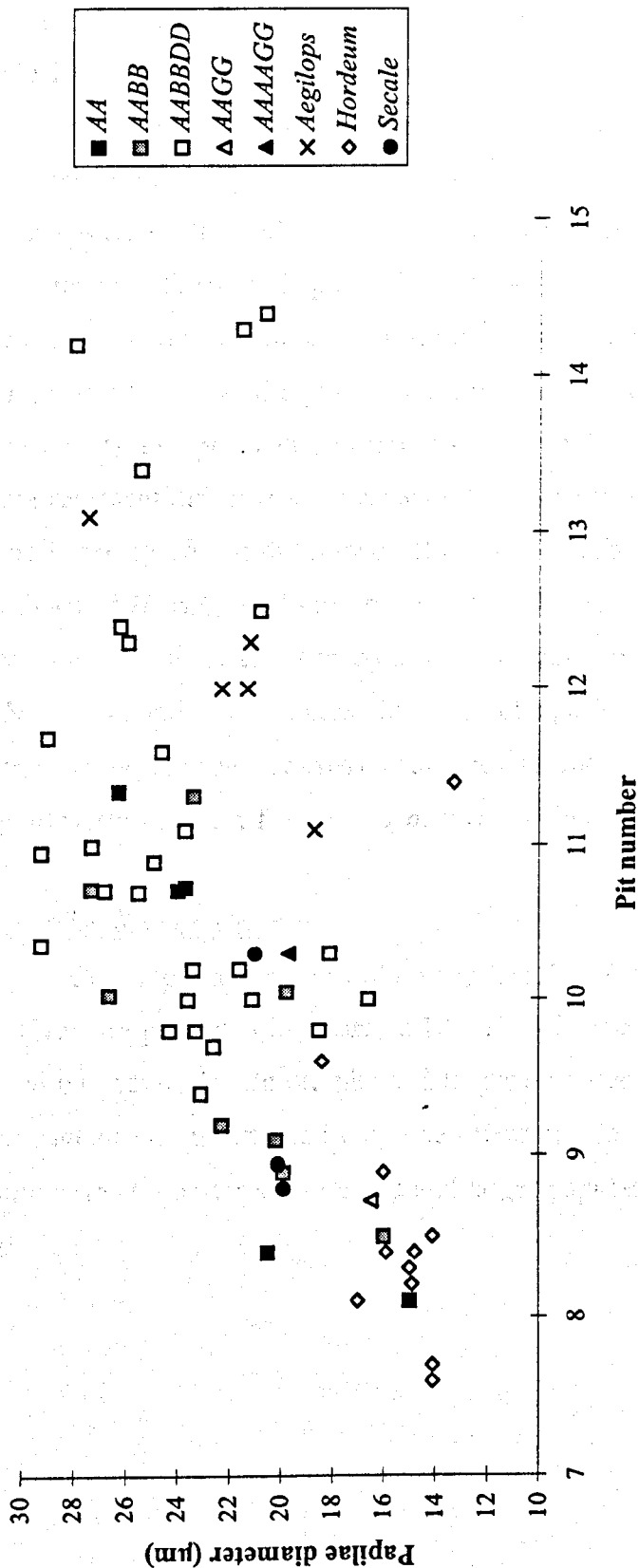




**Figure 2.7.** A comparison of average overall papilla diameter (in  $\mu\text{m}$ ) and pit number between species. The letters after *Triticum* indicates genomic designation. *Hordeum* represents the mean of all *Hordeum* species investigated and *Aegilops* represents the mean of all *Aegilops* species investigated.



**Figure 2.8.** A comparison of average overall pit number and papillae diameter (in  $\mu\text{m}$ ) between *T. aestivum* cultivars. UK indicates wheat from the United Kingdom, Can indicates wheats from Canada, old/new indicates age of formation of cultivar and red/white indicates type of wheat.



**Figure 2.9.** Scatter diagram of papilla data from all accessions/species, plotting mean overall (glume plus lemma) number of pits against papilla diameter. *AA* represents wheats with *AA* genome. *AABB* represents wheats with *AABB* genome. *AABBDD* represents wheats with *AABBDD* genome, including *T. aestivum*. *AAGG* represents wheat with *AAGG* genome. *AAAAGG* represents wheat with *AAAAGG* genome.

*Aegilops* sp., *Triticum* sp. AA, *Triticum* sp. AABB and *Triticum* sp. AABBDD was significantly different between all the groups except papilla diameter between *Triticum* sp. AA and *Triticum* sp. AABB groups (see table 2.6).

Overall pit number and papilla diameter for each species studied was compared statistically (by t-test) to each other in a 70 x 70 matrix. All species were significantly different from each other either by pit number or diameter or both ( $p=0.05$ , pit number: d.f.=398, diameter: d.f.=198, except for tests involving *H. jubatum*, in these cases pit number: d.f.=298, diameter: d.f.=148 as the glumes were reduced to awns and were not considered), except for those presented in table 2.7. Shaded areas in the table represent different species (with different genomic designation) where there is no significant difference. It can be seen for the table that various cultivars of *H. vulgare* do not possess significantly different papillae parameters. This is also observed for some cultivars of *T. aestivum*. *T. boeoticum* and *T. boeoticum* (2) were not significantly different. Interestingly, the specific cross *T. dicoccum* x *Ae squarrosa* was not significantly different from two *T. aestivum* cultivars.

#### Data from Known Crosses.

Crossing diagrams including papillae data for all the individuals involved are presented in figures 2.10. The offspring from each cross had, based on overall data, more pits and larger diameter papillae than its *Triticum* species parent, and only one offspring, *Ae. speltoides* x *T. monococcum*, possessed more pits and larger papillae than its *Aegilops* parent.

**Table 2.6.** The results of T-tests between groups of species studied, the values in the table are calculated T values.

**Table 2.6a)** Overall papilla pit number.

	<i>Triticum</i> AABBDD	<i>Triticum</i> AABB	<i>Triticum</i> AA	<i>Aegilops</i>
<i>Hordeum</i>	67.5 <sup>1</sup>	26.0 <sup>2</sup>	14.9 <sup>3</sup>	74.3 <sup>3</sup>
<i>Triticum</i> AABBDD	X	33.2 <sup>4</sup>	34.5 <sup>5</sup>	18.9 <sup>5</sup>
<i>Triticum</i> AABB		X	6.29 <sup>5</sup>	45.6 <sup>5</sup>
<i>Triticum</i> AA			X	45.8 <sup>6</sup>
<i>Aegilops</i>				X

**Table 2.6b)** Overall papilla diameter.

	<i>Triticum</i> AABBDD	<i>Triticum</i> AABB	<i>Triticum</i> AA	<i>Aegilops</i>
<i>Hordeum</i>	66.0 <sup>7</sup>	31.5 <sup>8</sup>	33.4 <sup>9</sup>	39.7 <sup>9</sup>
<i>Triticum</i> AABBDD	X	11.9 <sup>10</sup>	12.1 <sup>11</sup>	6.61 <sup>11</sup>
<i>Triticum</i> AABB		X	0.288 <sup>12</sup>	4.78 <sup>12</sup>
<i>Triticum</i> AA			X	4.63 <sup>13</sup>
<i>Aegilops</i>				X

1.d.f.= 7498  
2. d.f.=3698  
3. d.f.=3098

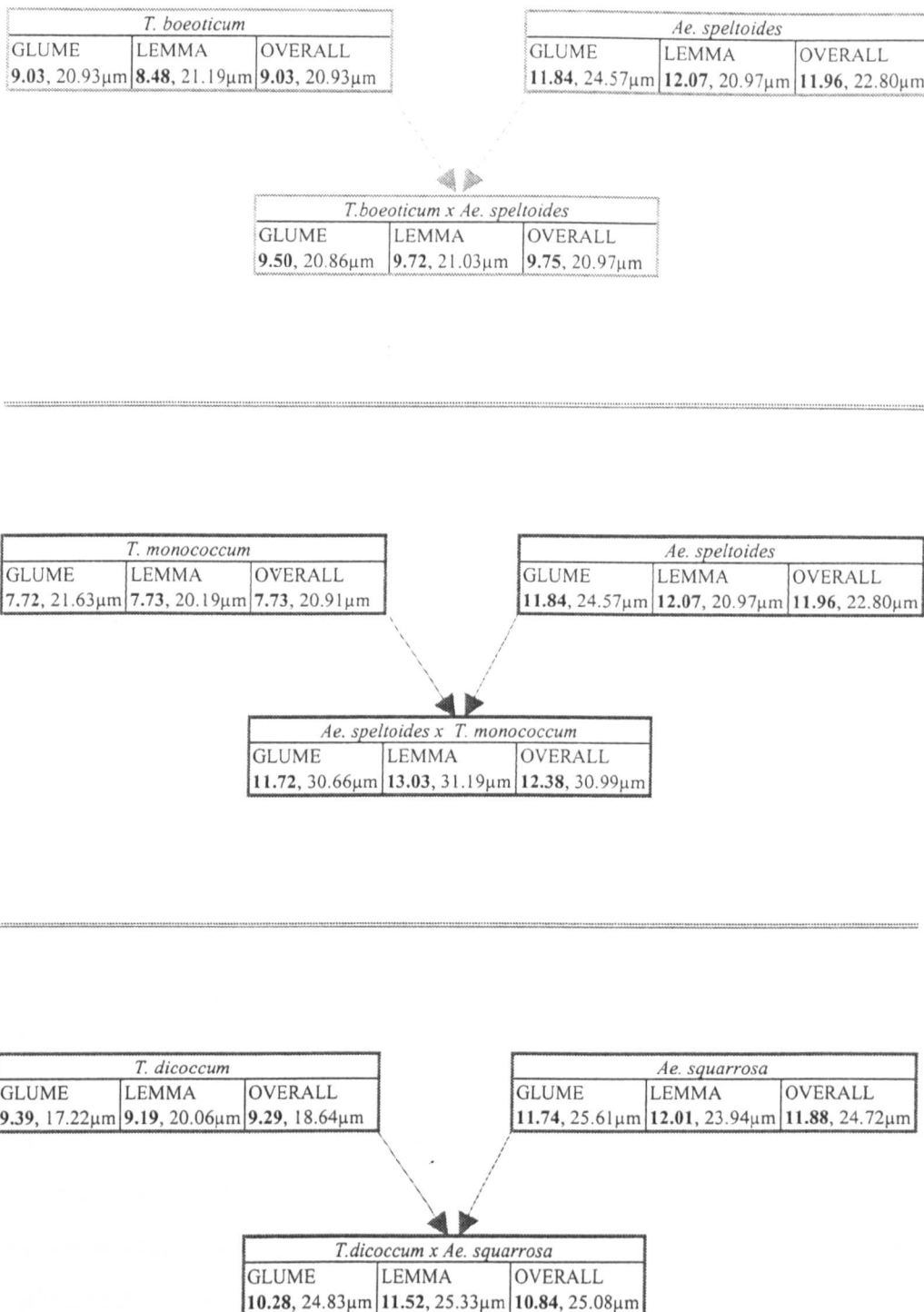
4. d.f.=6998  
5. d.f.=2598  
6. d.f.=1998

7. d.f.=3748  
8. d.f.=1848  
9. d.f.=1548

10. d.f.=3498  
11. d.f.=3198  
12. d.f.=1298  
13. d.f.=998

**Table 2.7.** A list of the species/accessions in the present investigation which showed no significant difference with regard to both pit number and diameter of papillae, based on overall mean averages. (d.f. =398, p=0.05). Shaded areas represent comparisons between species/accessions which do not possess the same genetic composition.

<i>Ae. speltooides</i> (2)	X	<i>Ae. squarrosa</i>
<i>H. vulgare</i> cv. 1098804	X	<i>H. vulgare</i> cv. Goldthorpe
<i>H. vulgare</i> cv. Plumage	X	<i>H. vulgare</i> cv. Bere
<i>T. aestivum</i> cv 175/768	X	<i>T. aestivum</i> cv 90/6
<i>T. aestivum</i> cv Browick	X	<i>T. aestivum</i> cv Little Joss
<i>T. aestivum</i> cv Highbury	X	<i>Ae. speltooides</i>
<i>T. aestivum</i> cv Houser	X	Triticale cv. Decade
<i>T. aestivum</i> cv Houser	X	<i>T. aestivum</i> cv Benoist 40
<i>T. aestivum</i> cv Perlo	X	<i>T. aestivum</i> cv Absolvent
<i>T. boeoticum</i> (2)	X	<i>T. boeoticum</i>
<i>T. dicoccoides</i>	X	<i>T. boeoticum</i> (2)
<i>T. dicoccum</i>	X	<i>Secale cereale</i>
<i>T. dicoccum</i> x <i>Ae. squarrosa</i>	X	<i>T. aestivum</i> cv 90/4
<i>T. dicoccum</i> x <i>Ae. squarrosa</i>	X	<i>T. aestivum</i> cv Frankenmuth
<i>T. monococcum</i>	X	<i>H. vulgare</i> cv. Goldthorpe
<i>T. monococcum</i>	X	<i>H. vulgare</i> cv FR 79393
<i>T. sinskaye</i>	X	<i>T. thaudar</i>
<i>T. timopheevi</i>	X	<i>H. murinum</i>
<i>T. vavilovi</i>	X	<i>T. polonicum</i>



**Figure 2.10.** The papilla data for crosses of known parents, pit number is the first number (in bold type), the second number is diameter in μm. Overall data is the mean of glume and lemma data.

## 2.4. Discussion.

Silicified papillae on the outer surface of the glume and the lemma of the inflorescence of wheats and barleys were viewed using light microscopy and scanning electron microscopy. Both methods yielded similar results with regard to pit number and papilla diameter. The glume and the lemma of each accession were ashed and analyzed separately and the results indicated that neither the diameter nor the number of pits in the papilla was consistently greater in either bract (Tables 2.2-2.5). For taxonomic work any differences between glumes and lemmas may be important, but differences are less critical in archaeology, where it will often be uncertain from which bract the papilla has originated.

A natural assumption is that as the papilla diameter increases there will be more available space for, or a requirement for, more pits, and hence the pit number would tend to increase although the results presented suggest there is no correlation between pit number and papilla diameter. The depressions (pits) in the papilla are thought to represent points of contact with the underlying cells (Hayward & Parry, 1980) and the micrographs presented in this chapter (fig 2.3a, b and 2.4a) support this suggestion.

Hayward and Parry (1980) observed a pit number of 7-9 in the inflorescence papillae of *H. vulgare*, as did Rosen (1992). The present work gave an average pit number for all *H. vulgare* cultivars of 8.2, which is in close agreement. The work on *Hordeum* species in this investigation must be regarded as preliminary as it was mostly confined to *H. vulgare* cultivars. However, the results showed that *H. murinum* papillae have a closer resemblance to *H. vulgare* cvs. than does *H. jubatum* (Fig. 2.6). *H. jubatum* papillae generally had a smaller diameter and a higher number of pits than *H. vulgare* and was similar to *H. secalinum*. Jørgensen (1986), using electrophoresis of six enzyme systems, placed *H. murinum* and *H. vulgare* in the "*H. vulgare*" group and placed *H. jubatum* and *H. secalinum* in the "pubiform" group (subgroup 4), indicating a closer similarity between *H. murinum* and *H. vulgare* than with *H. jubatum* and *H. secalinum*. Thus the similarity of the papilla parameters may give an indication of the degree



of relatedness between *Hordeum* species.

It is also apparent that in *H. vulgare* the more modern cultivars have larger papillae than the older cultivars (fig. 2.6). It is possible that the increased crossing and intensive breeding of the modern cultivars is affecting papilla size. Among other characteristics, breeders also select for pest resistant plants, and silica has been implicated as a barrier to pests (see section 3.1). Therefore, resistant plants may deposit silica more heavily and have larger papillae.

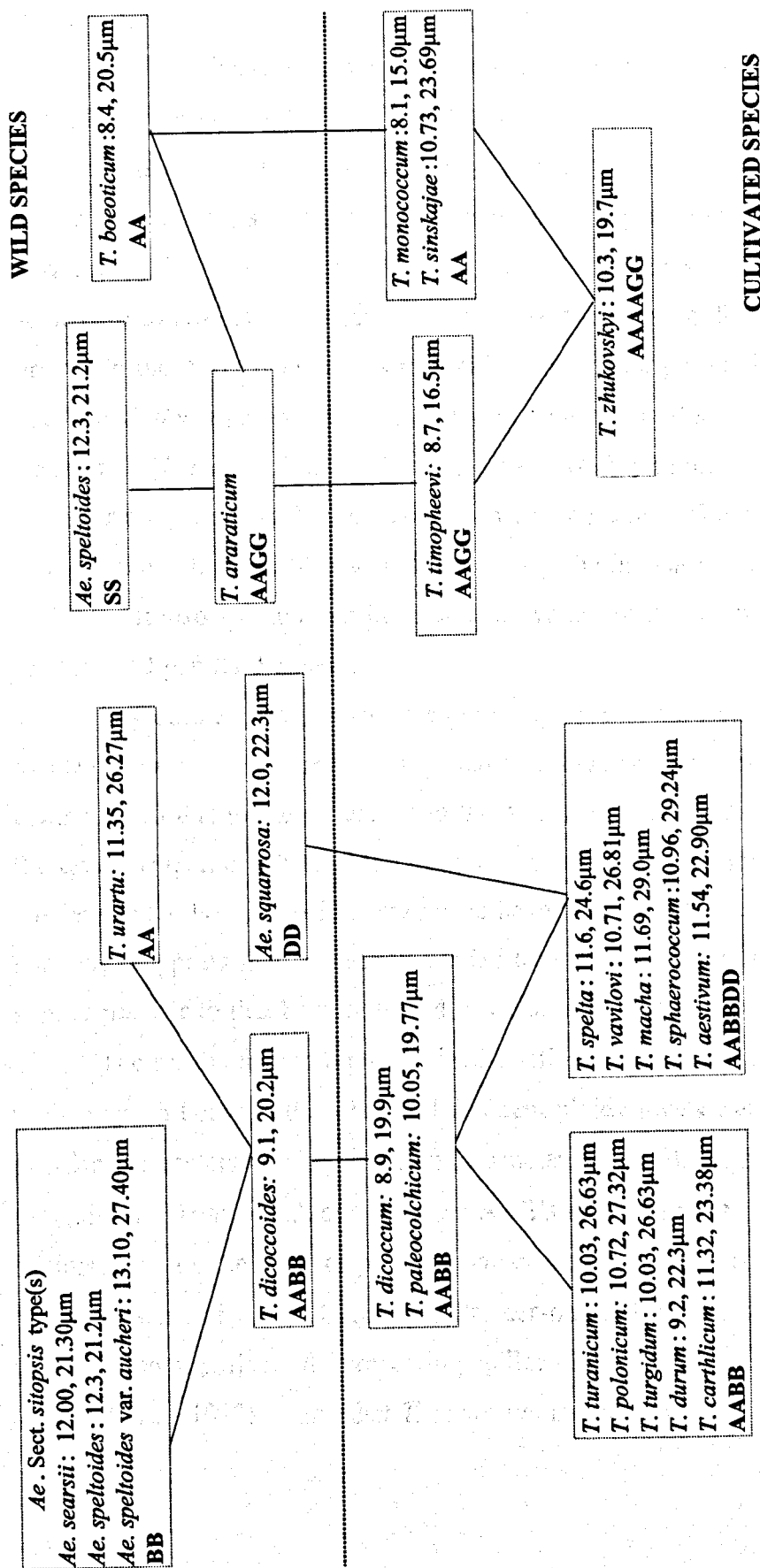
The data presented for rye is very limited with only one cultivar of *Secale cereale* being analyzed, although 4-12 species have been recognized in the genus of *Secale* (Stutz, 1972). The pit number and/or papillae diameter of this rye cultivar was significantly different compared to all the other species of this investigation except *T. dicoccum*. This may be a problematic overlap and further investigation is needed into rye papillae parameters.

Triticale (x *Triticosecale* Wittmack) is an amphiploid (i.e. consists of the somatic genetic complement of both parents) of wheat and rye. Decaploid, octoploid, hexaploid and tetraploid triticale can all be produced (reviewed by Gupta & Priyadarshan, 1982), although most research has been directed towards tetraploid triticale (Gregory, 1987). This is produced by crossing *T. durum* (genomic designation AABB) and rye, and typically wheat is the female parent (Larter, 1976). The papillae pit number of the triticale in the present investigation were significantly greater than that of rye. Pit number and diameter were similar to *Triticum* species AABB, and triticale cv. Decade showed no significant difference to the *Triticum* AABB group.

*Triticum* and *Aegilops* genus are closely related, indeed *Aegilops* as a separate genus has been disputed (see Miller, 1987). In the present work, the papillae of the two genera appeared similar, with a flattened rim and the pits in full view. *Aegilops* papillae, on average have significantly higher pit number than any *Triticum* species AABBDD (12.1 and 11.1, respectively), and smaller papilla diameter (*Aegilops* 22.6  $\mu\text{m}$ ; *Triticum* AABBDD 27.8

μm; tab. 2.6). However, most *Aegilops* species analyzed were from the section Sitopsis, and do not represent the whole of the *Aegilops* genus. *Ae. squarrosa* is a member of the section Vertebrata and is closely related to *T. aestivum*, while *Ae. umbellulata*, which is a member of the Polyeides section is not as closely related, and its papilla parameters are less like *T. aestivum*. Rosen (1992) measured papilla diameter and pit number in *Ae. searsii* as 25-27 μm and 16-18, respectively. The results in the present investigation were  $21.3 \pm 2.8 \mu\text{m}$  for papilla diameter and  $12.00 \pm 1.4$  pit number. Therefore this species appears to show some variation in papillae parameters and further analysis of *Ae. searsii* accessions are required.

The results presented demonstrated that as the ploidy level of wheat increased so did the papilla pit number and diameter (fig. 2.7) therefore, an attempt was made to relate this data to wheat evolution (fig. 2.11). Wheat evolution is complex and has been reviewed by Percival (1921), Kimber & Athwal (1972), Ford-Lloyd & Jackson (1986), Hilu (1987), Kahler & Price (1987), Kimber & Sears (1987), Miller (1987) and Hancock (1992). The species of wheat are either diploid (AA), tetraploid (AABB or AAGG) or hexaploid (AABBDD or AAAAGG). The genomic formula for *Triticum* species was given in table 2.4 and 2.5. Hybridization of AA wheats with the B or G genome donor produced the tetraploid wheats. The exact species which donated the B or G genomes is unknown, although various species have been suggested for the B donor, such as *Agropyron triticeum*, *Ae. bicornis*, *Ae. speltoides* and *Ae. searsii* (see Miller, 1987). Graur, Bogher & Breiman (1989), using mitochondrial restriction endonuclease profiles, suggested that *Ae. longissima*, *Ae. speltoides*, *Ae. bicornis*, *Ae. sharonensis* and *Ae. searsii* were not likely to have donated the B genome. It is possible that the B donor is an extinct or undiscovered species or the genome was derived from more than one source, alternatively DNA rearrangement within tetraploid wheat may have occurred altering the B genome (Miller, 1987). Similar controversy surrounds the G genome donor and the species suggested as the B genome can also be suggested as G genome donors. AABB diploids show similarities to *T. urartu* (AA)



**Figure 2.11.** The evolution of the polyploid wheats (adapted from Miller, 1987) with papillae data from the present investigation added after each species. The first number is overall pit number followed by overall papilla diameter, in µm.

whereas AAGG wheats are similar to *T. boeoticum* and *T. monococcum*, thus suggesting alternative A genome donors to these diploids. *T. zhukovskyi* is the only AAAAGG hexaploid wheat and this an amphiploid of *T. monococcum* and *T. timopheevi* (fig. 2.11).

AABB wheats hybridized with the D genome donor to form hexaploid wheats, AABBDD. The D genome donor is *Ae. squarrosa*. *T. dicoccum* is thought to be the maternal parent of these wheats, although Graur *et al.* (1989) suggested *T. turgidum*. By comparing the papillae parameters, from the present investigation, of *T. dicoccum* and *Ae. squarrosa* to AABBDD wheats it appears that the papillae diameter is greater in the AABBDD wheats than either parent and pit number is greater than the *Triticum* parent. This is also seen with the results for *T. zhukovskyi* (*T. monococcum* x *T. timopheevi*). Both pit number and papillae diameter are greater in *T. zhukovskyi* than either parent. This could be due to increased cell size which would accompany the increase in genetic material. It also appears that increased cultivation of AABB wheats has led to increased papilla diameter.

Phytolith analysis of teosinte (*Zea mays* subsp. *parviglumis*) and maize (*Zea mays* subsp. *mays*) suggested that these two species could be distinguished due to the difference in the size of the phytoliths produced in the leaves (Piperno, 1984). Maize phytoliths are larger than those of teosinte and although teosinte and maize have the same chromosome number, it appears that cultivation has led to increased phytolith size in a similar manner to that have observed in wheat in the present investigation.

The results from the present investigation suggest that it is possible to distinguish between the wheats of different ploidy levels using the papillae parameters of pit number and diameter (tab. 2.5). The only exception is *Triticum* AA and *Triticum* AABB which can not be distinguished on the basis of papillae diameter only. This is consistent with results presented by Ball, Gardner & Brotherson (1995), who suggested that there was no significant difference in papillae dimensions.

Rosen (1992), found that *T. dicoccum* had papillae with a diameter

of 22-30  $\mu\text{m}$  (somewhat larger than the present accession) and a pit number of 10-12 (again a little larger). However, her *T. dicoccoides* accession had a 21-43  $\mu\text{m}$  papilla diameter and a 16-18 pit number. The results obtained currently were 20.2  $\mu\text{m}$  and 9.2 (respectively). Dr. Rosen kindly provided ash from the inflorescence bracts of her *T. dicoccoides* and *T. dicoccum* accessions. Analysis of this material agreed closely with her results for both species (data not presented). The initial accession of *T. dicoccoides* examined in the present study was obtained from the Samarian Mountains, Israel (via the Hebrew University and the John Innes Institute), while Rosen's accession was from the Gilat Agricultural Station in the Northern Negev Desert, Israel. The papilla differences may be due to genetic variation, and *T. dicoccoides* is well known to be a very variable species. For example, Nevo, Gorham and Beiles (1992), showed that there was considerable variation in sodium-22 uptake in accessions of *T. dicoccoides* isolated from a number of areas in Israel. It is possible that the papilla parameters vary in a similar way, possibly due to variations in silica uptake. In the current investigation, *T. aestivum* also has great variation between cultivars. Papilla diameter for 13 cultivars of *T. durum* was analyzed and compared to the data in this section of thesis for *T. aestivum* cultivars (Westerman & Hodson, 1994). The results suggested that *T. durum* cultivars had smaller diameter papillae than *T. aestivum*, although there was some overlap, however pit number was not measured. Further work will be required to establish the full range of variation within this species. There is also discrepancy between papillae parameters reported by Rosen (1992) for *T. monococcum* (diameter=20-30  $\mu\text{m}$ , pit number=12-14) and that of the current investigation (diameter=  $15.00 \pm 2.9$ , pit number=  $8.10 \pm 1.2$ ). This highlights the need for further analysis of more accessions.

Considering the cultivars in the present investigation, *T. aestivum* has significantly larger papillae than most, but not all, *H. vulgare* cultivars (tab. 2.6). However, pit number proved to be the best means of distinguishing between the two species, as all of the *T. aestivum* cultivars analyzed had significantly more pits than any *H. vulgare* cv.. The papillae

from wheat are structurally different from those of barley. Barley papillae have a rim on the base which obscures the pits, whereas in wheat papillae the rim is flattened out so that it is in line with the epidermis, allowing full view of the pits in the base (compare Figs. 2.3d and 2.4b). This may be another diagnostic difference between wheat and barley. *T. aestivum* also shows increasing papilla diameter with decreasing age of the formation of the cultivar similar to *H. vulgare* cvs.

The result of the papilla analysis carried out on known hybrid crosses (fig. 2.10) clearly showed that the offspring had a higher pit number than its *Triticum* parent. The offspring do not show any consistent pattern of similarity to either parent and therefore it is difficult to determine if there is any genetic control over papilla parameters. An interesting result from these crosses was that of *T. dicoccum* x *Ae. squarrosa*. This cross is considered to have occurred, during wheat evolution to produce *T. aestivum*. In the present investigation the offspring produced from the above cross was not significantly different from two of the present *T. aestivum* cultivars (90/4 and Frankenmuth), and interestingly these are both "new" cultivars.

A further interesting result was that there was no significant difference between *T. boeoticum* and the offspring grown from the grain of these individuals, termed *T. boeoticum* (2). This suggested that environmental factors did not affect papillae parameters and is consistent with work by Ball & Brotherson (1992) who also reported that phytolith morphology was not significantly affected by the environment. However, Rosen & Weiner (1994) reported that irrigated *T. turgidum* produced phytoliths with greater numbers of silicified cells cemented together than dry-farmed wheat.

The present analysis of one type of phytolith in a range of plants shows a significant difference between *H. vulgare* and *T. aestivum*. This would allow an archaeologist to distinguish between these two species on the basis of papilla analysis alone. The increasing papilla diameter and pit number with increasing ploidy level in *Triticum* species may be useful in determining the ploidy level of wheat being grown and utilized at a

particular time. This can be a problem in archaeological remains (Kislev, 1984). In the present work, the progenitor *Triticum* species analyzed were modern forms of ancient wheats. During cultivation these crops may have evolved and papilla morphology may have changed over time. Therefore it may be necessary to analyze papillae from archaeological remains of known species, to establish if any such change has occurred.

Some work has been done on the dendriform cells and other phytoliths (Rovner and Russ, 1992), and they may also contain information. If papilla analysis could be combined with analysis of these structures it may allow greater accuracy in identifying plant species from microfossils. The present study identified some species which were not significantly different with regard to papillae parameters (see table 2.6). Finding no significant difference between cultivars of the same species is of little concern. If this occurs between different species these other phytoliths may be useful. Papillae occur on the inflorescence bracts of other plant species and these may overlap with the wheat and barley results, thus making it impossible to distinguish plant species on papilla data alone.

## CHAPTER 3: THE BENEFITS OF SILICA.

### **3.1. Introduction.**

Silica, mostly in the deposited form, has been observed in numerous plant species (for examples see table 2.1, chapter 2). Although silica is often referred to as a "beneficial" plant nutrient, its possible function(s) within the plant remains elusive. The potential roles of silica in plants have been reviewed by Lewin & Reimann (1969), Werner & Roth (1983), Cheng (1982), Takahashi, Ma & Miyake (1990) and Epstein (1994).

#### Possible Roles for Silica.

The proposed functions of silica are numerous and wide ranging. Ponnamperuma (1964) reviewed the effect of silica on rice plants stating that silica increased resistance to diseases and insect pests, promoted erect growth habit and increased the oxidizing power of rice roots. Elawad, Gescho & Street (1982) stated "the vital role of Si in sugarcane growth was evident by increased plant size and increased tillering". Silica has also been shown to have an effect on the reproductive growth in tomato plants, cucumber, soybean and fruit production in strawberry plants (Miyake & Takahashi, 1978, 1983, 1985, 1986). However Marschner *et al.* (1990) reported that these effects may be due to zinc/phosphate imbalances in the nutrient solution. More specific roles for silica include the possibility that silica is utilized, perhaps in addition to lignin, as a strengthening material (Kaufman *et al.*, 1972; Sakai & Thom, 1979; Soni, Kaufman & Bigelow, 1970; Postek, 1981). Raven (1983) suggested that silica was a more energetically favourable compound when equated to lignin, but emphasized silica would add greater weight to the plant.

Silica may possibly be involved in drought resistance (Ponnaiya, 1960), and other reports have suggested a role in water conservation. This could either be by reducing transpirational water loss, as silica can form an impermeable layer on leaf surfaces (Postek, 1981; Yoshida, Ohnishi, Kitagishi, 1962) or by hydration of the silica gel (Kaufman *et al.*, 1972). In leaves, an hypothesis put forward was that the deposits formed "light



windows/pipes" within surface cells which allowed light to penetrate to underlying cells and thus increase the photosynthetic capability of the plant, although this has since been rejected (see Kaufman *et al.*, 1985).

Temperature regulation has also been proposed as a feasible role (Kaufman *et al.*, 1985). Further to this, McWhorter & Paul (1989) suggested that silica cells create a local reduction in temperature which promotes the formation of wax filaments in johnsongrass plants. A more species specific role, perhaps, was the suggestion of Boylston *et al.* (1990), that silica may have a role in fibre development in cotton. Alternatively, it was proposed that silica deposits, in palm leaves, favour supercooling by the increased rigidity of the cell walls and/or resistance to the growth of ice crystals (Larcher *et al.*, 1991).

#### Herbivore Resistance.

The above speculative roles for silica deposits within plants have received limited attention compared with the possibility that silica may protect against herbivores and pathogenic fungi. Herbivore resistance is supported by the observation that grasses from heavily grazed areas contain more silica than grasses from areas of light grazing (McNaughton & Tarrants, 1983; Cid *et al.*, 1989; Brizuela, Detling & Cid, 1986; McNaughton *et al.*, 1985). However, the grazing preference of sheep was not related to grass silica levels (Shewmaker *et al.*, 1989). Conversely, prairie voles (*Microtus ochrogaster*) consumed less grass of high silica content than grass of low silica content (Gali-Muhtasib, Smith & Higgins, 1992), although the authors stated nitrogen levels in the plants were important in determining grazing preferences. Silica is intimately associated with the defense mechanism in stinging nettles (*Urtica dioica*) as the apical cell wall of the sting emergences consists of silica (Thurston, 1974), forming a brittle hair. The reported literature suggests that invertebrate herbivores are more affected than vertebrate herbivores by silica levels in plants: dipterous stem-borers, *Oscinella frit*, (Moore, 1984); slugs, *Agriolimax reticulatus* (Wadham & Parry, 1981); white-backed

planthoppers, *Sogatella furcifera* (Salim & Saxena, 1992). It is thought, therefore, that silica, as a defense mechanism, is more important in deterring invertebrate herbivores than vertebrate herbivores (O'Reagain & Mentis, 1989; Vicari & Bazely, 1993).

#### Fungal Resistance.

A large amount of data has been published concerning the effect of silica on plant resistance to fungal attack. In numerous studies, enhancing the growth medium of the plant with silica has led to an increased resistance to pathogenic fungi. For example silica supplements reduced powdery mildew attacks in wheat (Leusch & Buchenauer, 1989), significantly reduced mortality, root decay and yield losses associated with *Pythium ultimum* infection of long English cucumbers (Chérif & Bélanger, 1992), reduced severity of powdery mildew attack on cucumber, muskmelon and zucchini squash (Menzies *et al.*, 1992) and significantly reduced the severity of husk discoloration, neck blast, sheath blight and leaf scale diseases when applied to upland rice plants (Winslow, 1992). The question of how silica enhances resistance to fungal infection remains open. It has been shown that silica is present in the "halo areas" around fungal penetration sites in barley and wheat leaves (Kunoh & Ishizaki, 1975) in regions of haustorium encasement, necrotic host cytoplasm and adjacent host cell walls in cowpea plants (Heath, 1981a) and localized in barley cells invaded by powdery mildew (Carver, Zeyen & Ahlstrand, 1987). Initially it was thought that silica deposits within the cell wall acted as a physical barrier to the fungal hypha (Volk, Kahn & Weintraub, 1958), and this may be partly responsible for resistance of grape plants to mildew in the presence of silica (Bowen *et al.* 1992). Indeed Heath (1981b) suggested that attack by *Uromyces phaseoli* var. *typica* was determined by its ability to prevent deposition of silica-containing compounds within the host. It was suggested that silica deposition was promoted by phenolics, which are released following cell death upon invasion, accumulating in the cell wall and entrapping silica from the apoplast of the plant (Koga *et al.*, 1988). In

addition to silica acting as a physical barrier, Heath & Stumpf (1986) suggested two other possible functions of silicified cell walls; 1) that they reduce material interchange between host and fungus so that lesser amounts of phenolics are produced, 2) that material flow to the haustorial mother cell that normally prevents premature senescence is restricted. Silica deposition was not detected at the site of *Pythium ultimum* penetration of cucumber (Chérif, Menzies, Benhamou & Bélanger, 1992), supporting the idea that silica deposition *per se* was not responsible for the enhanced fungal resistance observed. Silica has been shown to reduce the initiation time for the production and/or the accumulation of phenolic compounds in host cells (an important host response mechanism), increase the number of cells involved (Menzies *et al.*, 1991; Chérif, Benhamou, Menzies & Bélanger, 1992) and stimulate chitinase activity and enhance activation of peroxidase and polyphenoloxidases (Chérif, Asselin & Bélanger, 1994). Samuels *et al.* (1994) suggested that the presence of silica within penetration sites "could be a rapid mechanism for modifying the cell wall during pathogen attack". The exact mechanism of silica enhancement of fungal resistance, however, remains unknown.

Silica deposits within the endodermis of the root have been suggested to enhance the barrier properties of this tissue layer in the root (Bennett, 1982) and this layer has been implicated in root pathogen resistance (see section 4.1).

#### The Effect on Nutritional Status.

Silica has been shown to affect the nutritional status in some plants, reducing manganese toxicity in various plants, by differing degrees (Williams & Vlamis, 1957 [barley], Vlamis & Williams, 1967 [barley, oats, rice, rye, ryegrass and wheat], Horst & Marschner, 1978 [*Phaseolus vulgaris*], Kluthcouski & Nelson, 1980 [soybeans], Galvez *et al.*, 1987 [sorghum] and Horiguchi, 1988 [rice]). In rice plants, silica appeared to decrease manganese uptake as well as enhance the internal tolerance to manganese toxicity (Horiguchi, 1988). Silica may also alleviate aluminium

toxicity in some plants (reviewed by Hodson & Evans, 1995) and salt stress (Bradbury & Ahmad, 1990; Ahmad, Zaheer & Ismail, 1992).

The above is a review of the possible roles of silica in plants. Many of these are concerned with plants under stress, such as attack by herbivores/fungi or nutrient imbalance. Very little data is available, however, concerning the effect of silica on growth parameters of unstressed plants, especially during the early stages of growth. Therefore, the aim of this section of thesis was to determine the effect of silica in a full nutrient solution on the growth of wheat seedlings. This was to be performed over the relatively short time period of 10 days. The effect was to be determined by measuring the growth parameters of root length, root and shoot fresh and dry weights and determining the shoot/root ratio of the wheat seedlings.

## 3.2. Materials & Methods

### 3.2.1. Plant Culture.

*Triticum aestivum* L. cv. Beaver was used for all the following experiments. This is a dwarf cultivar and was used because of space limitations within the growth cabinet, if it became necessary to grow the plants to maturity. Seeds (obtained from E. W. King & Co. Ltd., Kelvedon, Essex, U.K.) were germinated on Whatman 40 ashless filter paper moistened with distilled water in Pyrex petri dishes in a Fison growth cabinet. The cabinet had a photoperiod of 18 hours at 23°C, a dark period of 6 hours at 15°C, and the relative humidity ranged between 40-50% during the experimental period. The light (supplied by fluorescence tubes) intensity ranged from 370-280  $\mu\text{Einsteins m}^{-2} \text{ sec}^{-1}$  from the centre to the side of the growth cabinet (mean reading was 330  $\mu\text{Einsteins m}^{-2} \text{ sec}^{-1}$ ,  $n=4$ , measured using LI-COR, inc., model LI-185B, quantum/radiometer/photometer). The readings were taken from the top of the water culture containers (see below).

After 4 days germination, the seedlings were transferred to plastic water culture containers filled with nutrient solution and supported in holes in the lid by foam bungs. Eight plants were grown per 2 litre pot. The nutrient solution contained:  $\text{KNO}_3$  3mM;  $\text{NH}_4\text{NO}_3$  0.22mM;  $\text{Ca}(\text{NO}_3)_2$  0.5mM;  $\text{KH}_2\text{PO}_4$  0.3mM;  $\text{MgSO}_4$  0.6mM;  $\text{MnSO}_4$  0.01mM;  $\text{CuSO}_4$  0.002mM;  $\text{ZnSO}_4$  0.002mM;  $\text{H}_3\text{BO}_3$  0.15mM;  $\text{NaCl}$  0.1mM;  $(\text{NH}_2)_8\text{Mo}_{27}\text{O}_{24}$  0.003mM;  $\text{FeNaEDTA}$  0.05mM (all chemicals were obtained from BDH, Lutterworth, Leicestershire and were AnalaR quality). The macronutrient concentrations were after Van der Vorm (1980) and micronutrients after Hewitt (1966). All stock solutions were kept refrigerated and renewed as required, about every 2 months.

When silica was required in the solution ( $\text{Si}^+$ ) it was added in the form of sodium metasilicate ( $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ , obtained from Sigma Chemicals, Poole, Dorset). This stock solution was made-up freshly in a plastic container prior to use. A silica concentration of 0.5 mM was used in the experiments (as reported by Epstein, Norlyn & Cabot, 1988) and all

nutrient solutions were set at  $\text{pH } 5.2 \pm 0.1$  using HCl or NaOH.

The water culture containers were covered (both pot and lid) in aluminium foil to provide dark conditions for the roots and to prevent algal growth. The nutrient solution was gently and continuously aerated and topped up as necessary using distilled water. The plants were grown in the growth cabinet under the same conditions as stated above until the plants were harvested, up to 10 days after being transferred to the containers. There was no consistent variation of the pH of the nutrient solution during the experimental period.

### 3.2.2. Measuring Growth.

The amount of root growth was determined by the change in the length of the seminal roots present after the 4 day germination period. The plants were removed from the petri dish, the seminal roots were measured and "tagged" using coloured cotton thread tied loosely around the basal area of the root. The plants were then transferred to the water culture containers as detailed above and grown for the required period of time in either a nutrient solution containing no added silica, (Si-) or a nutrient solution containing 0.5 mM silica (Si+).

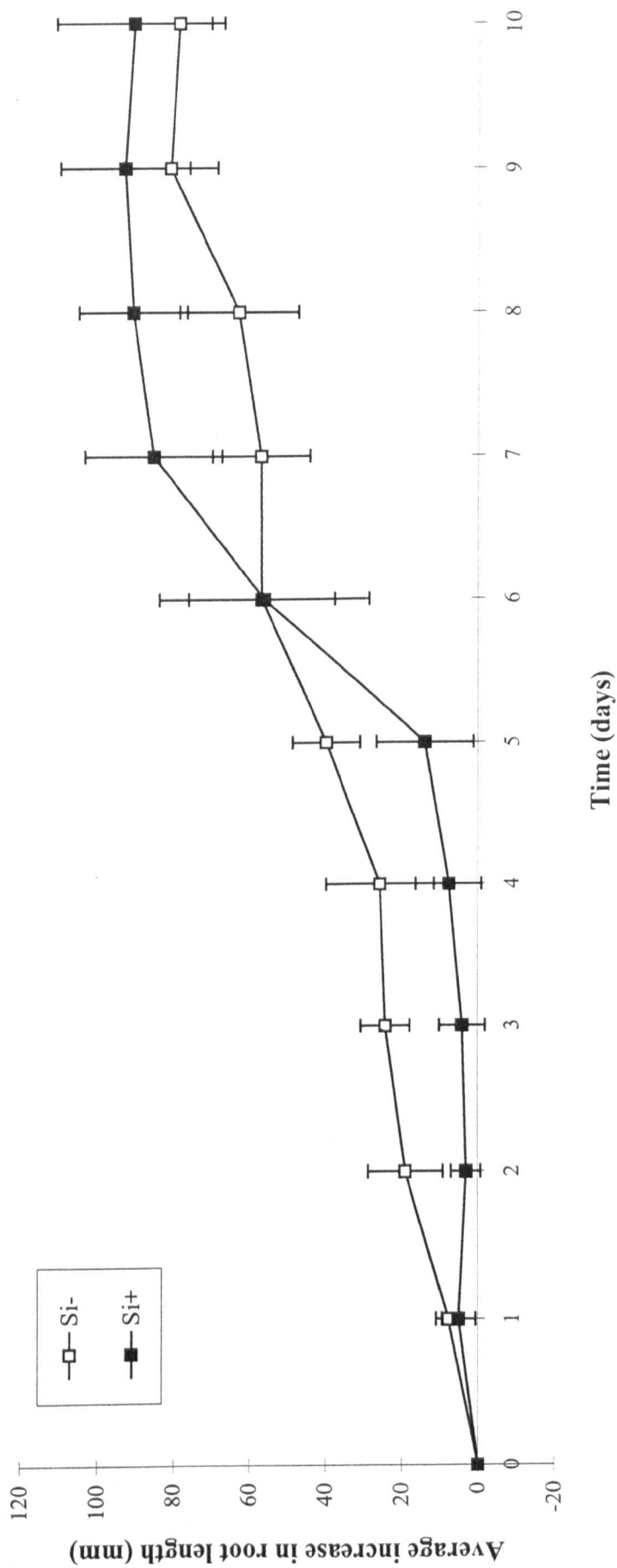
After the required time the plants were removed from the pots and the length of the "tagged" roots was recorded. Fresh and dry weight was determined by weighing the total root system and shoots separately before and after 48 hours in a 60°C oven in nickel crucibles. Details of the number of replicates are presented in the results section. Fresh and dry weight data was measured for the total number of plants and not individual plants.

### 3.3. Results.

#### 3.3.1 Root Growth.

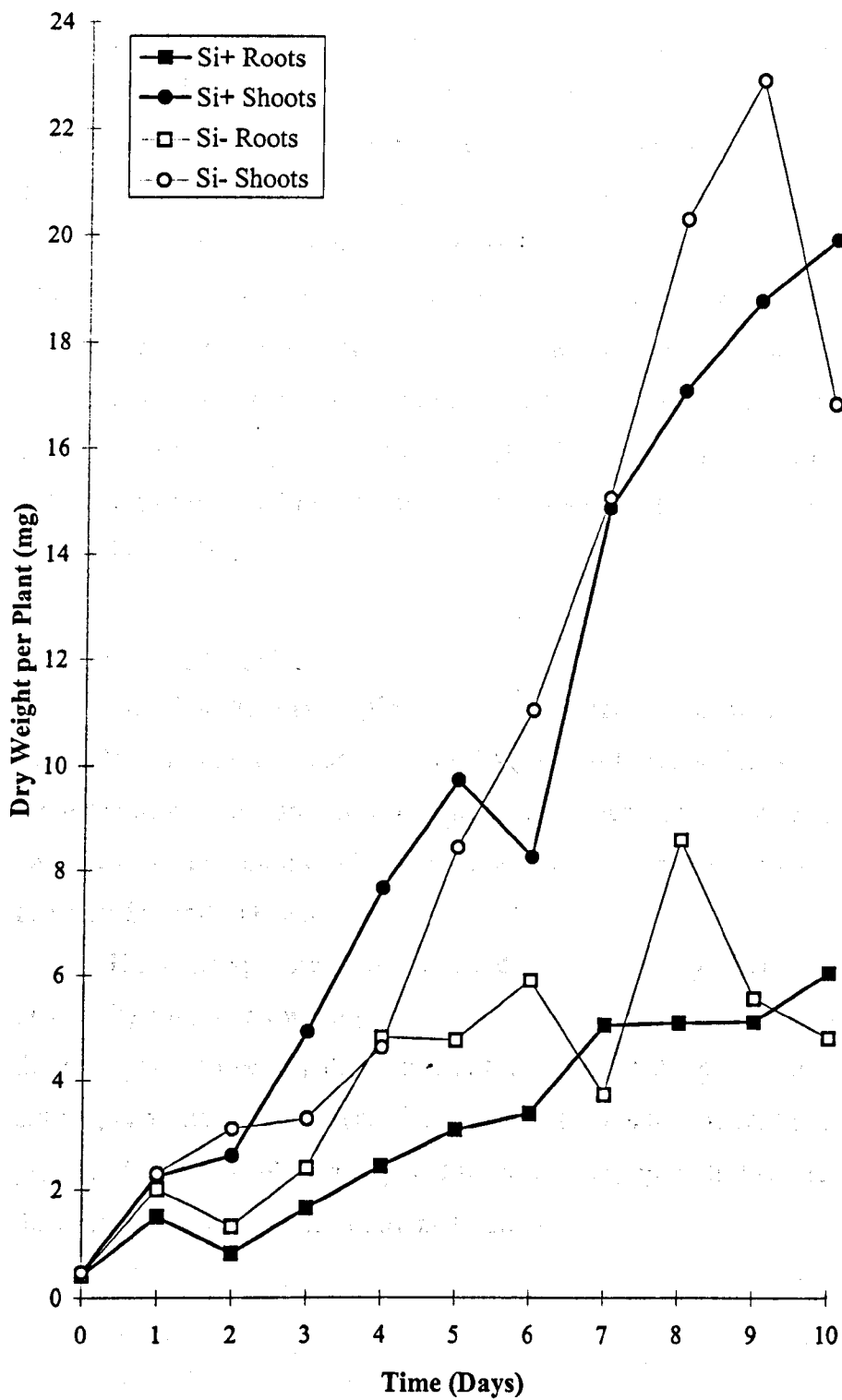
Figure 3.1 shows the mean average increase in root length of *Triticum aestivum* seedlings over a 10 day growth period in either Si+ or Si- nutrient solution. Over the growth period of 1 to 5 days, Si- plants displayed significantly greater increase in root length than Si+ plants (day 1,  $t=-3.78$ , d.f.=117; day 2,  $t=-11.58$ , d.f.=116; day 3,  $t=-17.85$ , d.f.=119; day 4,  $t=-8.52$ , d.f.=117; day 5,  $t=-10.47$ , d.f.=108;  $p=0.05$ ). Roots in Si- solution extended from 7.7 mm to 39.7 mm over the 5 days, whereas Si+ roots only extended from 5.07 mm to 13.9 mm over the same period. Between days 5-7 Si+ plant roots length increased rapidly, more so than Si- plant roots. After day 7, there was little increase in root length, in either Si+ or Si- roots until the termination of the experiment. The plants in Si+ solution have significantly longer roots ( $p=0.05$ ,  $t=23$ ,  $n=60$ ) than the plants grown in the Si- solution after a 10 day growth period. The roots of the Si+ plants increased 90.2 mm and the roots of Si- plants increased 78.4 mm over the growth period. Average root length data is presented in appendix I, table 1, (this data is required for calculations presented in section 4.3.6).

Dry weight (DWT) data is presented in figure 3.2. The dry weight of roots (mg) per plant increases linearly with time grown in the nutrient solutions. Over most of the growth period roots grown in Si- nutrient solution have greater dry weight than roots grown in Si+ solution. On day 1, Si- plants root DWT was 2.005 mg/plant and Si+ root DWT was 1.490 mg/plant. This difference was greater at day 6 where the DWT/plant had increased to 5.900 mg/plant for Si- plants and to 3.385 mg/plant for Si+ plants. When comparing this to fig. 3.1, this was to be expected in the earlier part of the growth period, days 1-6, as the roots are longer in Si- nutrient solution at this time. Fresh weight (FWT) data is presented in appendix I, table 2, (this data is required for calculations presented in section 4.3.6).



**Figure 3.1.** The increase in seminal root length (mm), relative to day 0, of wheat seedlings over a 10 day growth period in water culture with 0.5 mM silica added (Si+) and without silica added (Si-), ( $\pm$ std.,  $n=60$ ).





**Figure 3.2.** The dry weight of the roots and shoots of plants grown in water culture with 0.5 mM silica added (Si+) and without silica added (Si-).

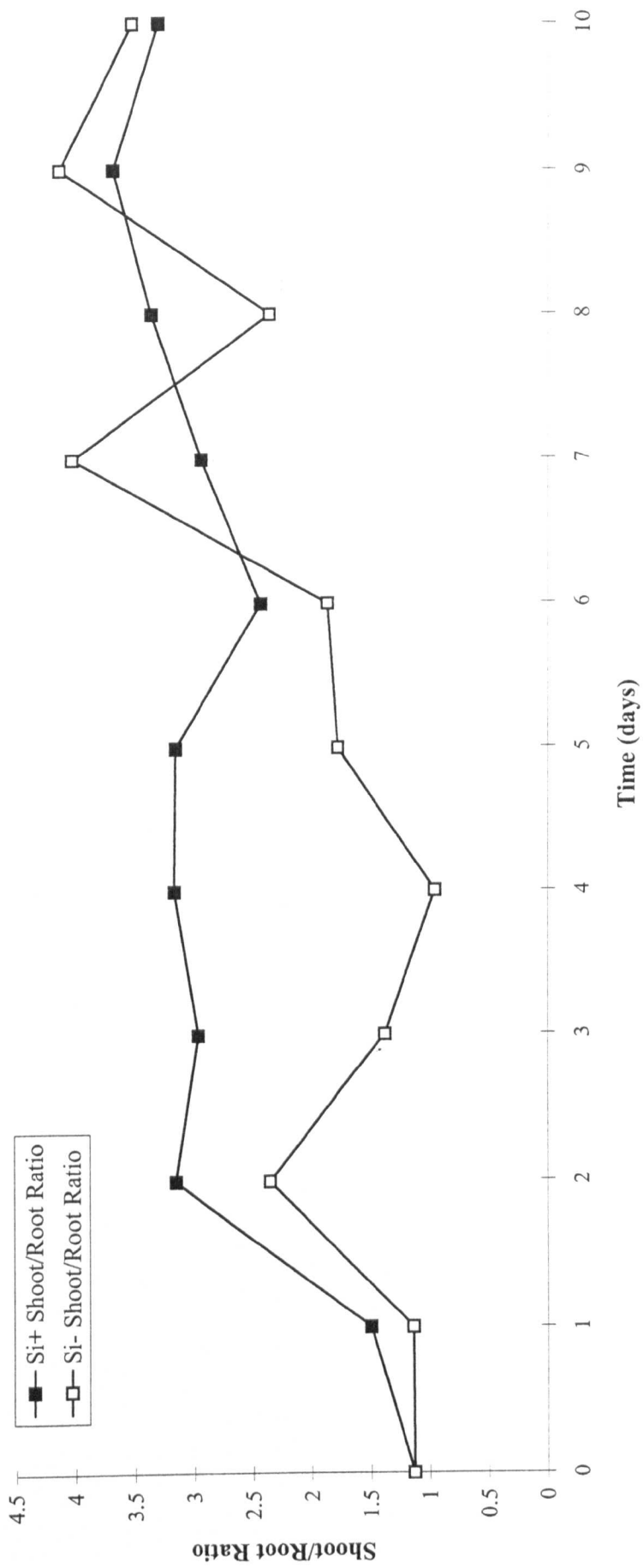
### 3.3.2. Shoot Growth

The DWT of the shoots of the plants also increases steadily over the 10 day growth period in plants grown in both Si- and Si+ solution (fig. 3.2). Initially the dry weight was very similar in both Si- and Si+ grown plants, (at day 1, 2.24 mg/plant in Si+ plants and 2.285 mg/day in Si- plants). Si+ plants had a slightly greater dry weight between day 3-6. Si+ plants had 8.270 mg/plant DWT on day 6 compared to Si- plants 11.050 mg/plants. However, at day 4 Si- plants show a slight increase in the rate of dry weight increase such that at day 8 - 10 Si- plants showed greater dry weight of shoots than did plants grown in Si+ nutrient solution. The fresh weight (FWT) data (presented in appendix I, table 2 this data is required for calculations presented in section 4) displays similar trends to that observed for the DWT data.

### 3.3.3. Overall Growth.

The shoot/root ratio (fig. 3.3) clearly demonstrates that during the first half of the experiment, Si+ and Si- plants differed strikingly in dry matter allocation to root growth. During the latter half of the experiment, day 6-10, there appears to be very little difference in the shoot/root ratio between Si- and Si+ plants.

The roots appeared healthy and did not show any obvious deficiency or toxicity symptoms when growing in Si- or Si+ nutrient solutions. There did not appear to be any difference in the shoots of the plants (fig. 3.4), although an interesting observation was that the containers that had plants growing in the Si- solution required more topping-up with distilled water than plants growing in Si+ nutrient solution.

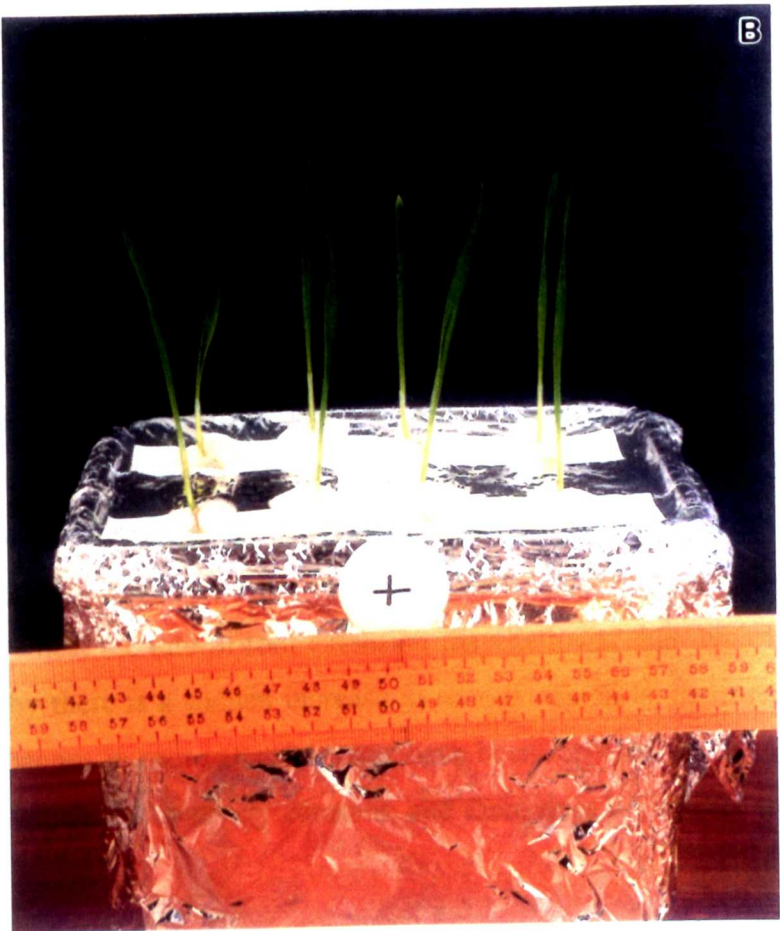
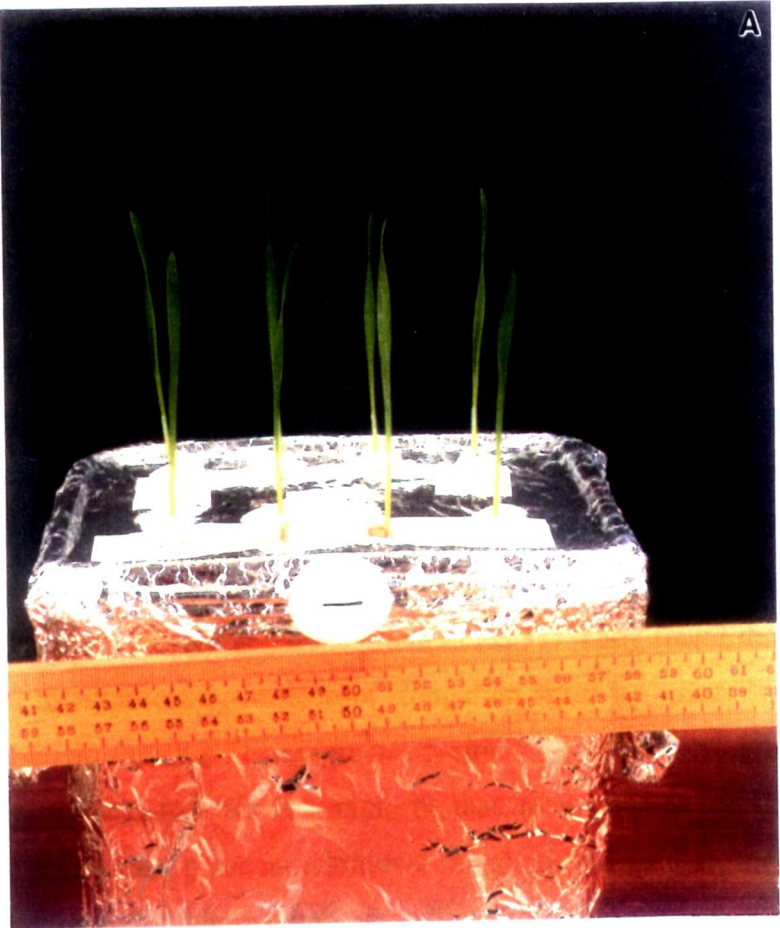


**Figure 3.3.** The shoot/root ratio of the dry weight of wheat seedlings over a 10 day growth period in water culture with 0.5 mM silica added (Si+) and without silica added (Si-).

**Figure 3.4.** The method of water culture is demonstrated. The wheat seedlings shown had been growing in the nutrient solution for 4 days, after a germination period of 4 days. There are 8 plants per pot containing 2 litres of full nutrient solution. The culture pots are covered with aluminium foil to exclude light. A central hole is provided in the lid of each pot (not clearly visible) for an air line to aerate and stir the solutions in the pot. The plants show no obvious signs of nutrient deficiency or toxicities. (The scale is centimetres).

A) Plants grown in full nutrient solution without added silica.

B) Plants grown in full nutrient solution with 0.5 mM Si added, as sodium metasilicate.



### 3.4. Discussion.

The aim of the present investigation was to determine the effect of silica on the growth parameters of root length, root fresh weight (FWT) and dry weight (DWT), shoot FWT and DWT and the shoot/root ratio.

#### 3.4.1. Root Growth.

Root extension is very variable and is affected by environmental factors such as nutrient supply (Russell, 1977). Therefore, the effect of silica supply on root extension was investigated. Initially, silica appeared to significantly depress the average increase in the length of the seminal root when compared to the Si- plants (fig. 3.1). A similar observation was also noted by Barcelo, Guevara & Poschenrieder (1993). They found that 4  $\mu\text{M}$  Si appeared to significantly lower root elongation when compared to Si- plants in *Zea mays* over a 21 day growth period (the plants were grown at pH 4). A possible reason for this might be that uptake and/or translocation of silica by plants may place an additional metabolic demand on the plant reducing root growth. Alternatively, the silica in the nutrient solution could be inhibitory to root extension, perhaps by affecting either the root meristem causing a reduction in cell production. A further possibility is silica may affect the cells of the elongation zone, possibly preventing cell wall extension or affecting water influx in to these cells (possibly by altering the osmotic potential of the nutrient solution). Emadian & Newton (1989), however, suggested silicon enhanced pine seedling growth by increasing cell expansion.

The results obtained for root extension are generally lower than typical values for cereals of 2.0 cm per day (Russell, 1977), except Si+ plants show an increase in growth rate on days 6 - 7 where rates of extension are 4.2 and 2.8 cm per day respectively. As Beaver is a dwarf cultivar of wheat the rate of root extension may be lower than typical values. Why there is a sudden increase in root extension is unclear, although changes in root extension rates have been reported under conditions which are relatively constant, and there does not appear to be

adequate basis for an explanation for such changes (Russell, 1977). Si+ plants may show a greater increase because at this point in their development they are responsive to silica stimulation. At the end of the experimental period Si+ plants have significantly longer roots than Si- plants.

As expected, the dry weight (DWT) of the total root system of both Si- and Si+ plants increases with time (fig. 3.2). When discussing weights of samples it should be remembered that the wheat cultivar, Beaver, is a dwarf wheat and therefore absolute values (not ratios) are not comparable to data from standard wheat cultivars. The Si- plants have a greater DWT of roots than Si+ plants over the whole experimental period of 10 days. This was expected over the first 5 days as the root length data indicates Si- plants have a larger root system than Si+ plants although, over a long term, a deficiency in nutrients reduces the total weight of both roots and shoots. Potassium and phosphorus deficiency reduce the dry weight of roots in barley (Hackett, 1968), however, this was due to a reduction in the nodal root system, not the seminal (the latter was investigated in this current section).

The present study compares well with a report by Okuda & Takahashi (1965). The authors found that although Si increased rice growth overall, during the first 10 days silicon slightly depressed growth compared to Si- plants. A slight increase in root DWT of rice when grown in 1.66 mM Si to full maturity has been reported (Ma & Takahashi, 1993). However, many studies report very little effect on root DWT when plants are exposed to silica. Neither mature ryegrass (*Lolium perenne*) or mature wheat displayed changes in root or shoot DWT when exposed to 0-0.7 mM (Jarvis, 1987). Also growth of *Bromus secalinus*, throughout the growing season was not affected by silica (Gali & Smith, 1992) in the range 0-100 mg/l Si (0-3.56 mM Si). These reported experiments were performed over long periods of time whilst the present section of thesis was concerned with the early growth stages of wheat seedlings in silica enriched solutions. Present results indicate a levelling off of the differences between Si- and

Si+ root DWT over time.

#### 3.4.2. Shoot Growth.

The DWT of the shoots of Si- and Si+ plants show very little difference over the experimental period except towards the end of the experiment on days 8-10 (fig. 3.2) when Si- plants had a greater DWT of shoots than Si+ plants. However, silica has been shown to increase shoot DWT in various plants, such as mature wheat (Ahmad, Zaheer & Ismail, 1992) loblolly pine (*Pinus taeda*, Emadian & Newton, 1989), rice plants (Ma & Takahashi, 1990) and strawberry plants (Miyake & Takahashi, 1986).

#### 3.4.3. Overall Growth.

The shoot/root ratio was investigated as this ratio is influenced greatly by factors such as nutrient levels, water, light, and temperature (Hunt & Burnett, 1973). The shoot/root ratio in the wheat plants grown showed an increasing investment in shoot DWT compared to root DWT (fig. 3.3). During the early part of the experiment, up to day 5, Si+ plants showed a greater proportion of dry matter was partitioned into the shoots than roots compared to Si- plants, although at the end of the experiment both show a similar shoot/root ratio. When compared to DWT data (fig. 3.2) it would appear that Si- plants do invest more in the root system than the shoot than Si+ plants. This may be due to silica enhancing nutrient uptake and therefore requiring a less extensive root system as discussed earlier. The addition of phosphate has been shown to cause marked differences in growth, and the root/shoot ratio is frequently depressed in ryegrass (Davidson, 1969). Additionally, in bean plants phosphorus deficiency caused a decrease in the shoot/root ratio (Cakmak, Hengeler & Marschner, 1994). Indeed, phosphorus, nitrogen and moisture levels also cause changes in root/shoot ratio, with the highest ratios occurring when there is an imbalance of these factors (Hackett, 1968). Therefore, the higher shoot/root ratio in Si+ plants may be due to an enhanced availability of



phosphate or an imbalance of nutrients (although data presented in section 5.3.5 did not show any significant difference in phosphate or potassium content of Si+ plants compared to Si- plants after 24 hours growth).

It would appear that roots are more affected than the shoots by the addition of silica. This is probably due to the fact that the roots are in direct contact with the silica and are probably controlling the quantity of silica which reaches the shoot. Early experiments demonstrated that a foliar application of 10 000 ppm Si (356.05 mM Si) severely retarded the growth of sugarcane and Si concentrations of 1000 ppm and 100 ppm (35.605 mM and 3.5605 mM respectively) reduced the fresh weight of the plants (Alexander, 1968). By applying the silica as a foliar spray any control by the roots was effectively bypassed and therefore allowing the effect of high silica on the leaves to be observed. It is possible, however, that the concentration of silica may be causing osmotic stress or the level of sodium in the salt supplied was approaching toxicity levels.

#### 3.4.4. Experimental Design Problems

There are problems with these comparisons between Si- and Si+ plants. The Si- plants do not have any additional silica added but silica contamination is very difficult to avoid (Werner & Roth, 1983). Likely sources of silica contamination are the chemicals used, distilled water (Mitsui & Takatoh [1963a] reported 0.005 ppm Si as  $\text{SiO}_2$ ), dust in the air and the surface of the seeds (Mitsui & Takatoh, 1963a; Islam & Saha, 1969). The problems of eliminating silica completely from a plants environment make it very difficult to determine whether it is an essential element or not for different plant species. Therefore, the comparisons made here are strictly between plants with a restricted supply of silica, Si- plants, and plants with available silica, Si+ plants. When comparing the growth of Si+ and Si- plants it should be remembered that the silica added was in the form  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ . Thus, when adding 0.5mM silica, 1mM sodium is also being added (in addition to the sodium already in the nutrient, 0.1mM NaCl, see section 3.2). However, when preparing Si- nutrient solution the

pH was lower than the required 5.2 and therefore NaOH was added to increase the pH, but this quantity was not recorded. It is thought that this does not affect the plants a great deal, if at all, as typically levels of sodium less than 100 mM are not considered to be saline conditions (Flowers & Läuchli, 1983). Epstein, Norlyn & Cabot (1988) grew *Zea mays* for 2 weeks, and then subjected some to 1 mM NaCl (Si- plants, control) and some to 0.5 mM Na<sub>2</sub>SiO<sub>3</sub> (Si+ plants). Therefore, the sodium levels were identical in the two treatments. They found that silica increased the shoot mean daily gain in fresh weight (Si-, 1.16 g, Si+ 2.50 g) and the root mean daily gain in fresh weight (Si-, 0.88 g, Si+, 1.49 g). They even suggested that silica minimal cultures were actually experimental artifacts.

The data presented demonstrates silica affects the growth parameters, especially of roots, of wheat seedlings when compared to plants with a reduced supply of silica. The observed effects appear to occur during the early stages of growth, typically up to 5 days after being transferred to the silica nutrient solution.

## CHAPTER 4: SILICA DEPOSITION AND SILICA POOLS.

### **4.1. Introduction.**

The possible functions of silica within plants were examined in section 3.1 and this current section reviews silica deposition and investigates silica partitioning. Silica deposits are ubiquitous in many plant species, occurring in inflorescence bracts (of grasses), leaves, stem/shoots (these deposits were reviewed in section 2.1) and subterranean organs. The deposits which are present in the roots of plants are the main focus of this chapter.

#### Root Deposits.

Silica deposition occurs in roots, and most studies have been carried out on grass roots. It can be seen from table 4.1 that the endodermal cells, and in particular the inner tangential wall (ITW) of these cells, has been shown to be the major site of silica deposition. Sangster & Parry (1976b) argued that "endodermal deposits represent a specialized aspect of silicification".

#### Endodermis.

A brief overview of endodermal structure and function is presented here, and more detailed information can be obtained from reviews by Van Fleet (1961), Clarkson & Robards (1975) and Luxová & Čiamporová (1992). The endodermis is a single cell layer between the root cortex and stele. During its development the cell wall is modified extensively by the incorporation of substances such as lipid, phenols and suberin (Wilson & Peterson, 1983). The primary stage of development occurs several millimetres from the root apex, and is characterized by the formation of a Casparian strip (Luxová & Čiamporová, 1992). This is a modified area in the radial wall of the cell. The Casparian strip consists of suberin and the plasma membrane adheres firmly to the cell wall at this point (Bonnett, 1968). This structure prevents apoplastic transport between the cortex and the stele (see section 5.1). The cell wall may then enter secondary

**Table 4.1.** Summary of silicification of root tissue.

Species investigated	Root Tissue Silicified	Authors
<i>Andropogon gerardii</i>	Endodermis (ITW, aggregates)	Sangster (1977)
<i>Andropogon scoparius</i>	Endodermis (ITW, aggregates)	Sangster (1977)
<i>Avena sativa</i> (adventitious roots)	Endodermis (ITW and radial walls)	Bennett (1982)
<i>Avena sativa</i> (seminal roots)	Endodermis (mostly ITW)	Bennett (1982)
<i>Hordeum vulgare</i> (adventitious roots)	Endodermis (all cell walls)	Bennett (1982)
<i>Hordeum vulgare</i> (seminal roots)	Endodermis (mostly ITW)	Bennett (1982)
<i>Miscanthus sacchariflorus</i>	Endodermis (ITW, aggregates)	Sangster (1983)
<i>Molinia caerulea</i>	Endodermis, stele, vessel walls and sub-epidermal sclerenchyma	Parry & Kelso (1975)
<i>Molinia caerulea</i>	Intercellular spaces of cortex, near endodermis	Montgomery & Parry (1979)
<i>Oryza sativa</i>	Endodermis (ITW and Radial walls)	Parry & Soni (1972)
<i>Phalaris canariensis</i> (adventitious roots)	Endodermis	Hodson (1986)
<i>Phalaris canariensis</i> (aerial adventitious roots)	Epidermis and cortex	Hodson (1986)

Table 4.1 continued.

Species investigated	Root Tissue Silicified	Authors
<i>Saccharum officinarum</i>	Endodermis (ITW) and endodermal/pericycle boundary (aggregates)	Parry & Kelso (1977)
<i>Sasa palmata</i>	All cell walls of the endodermis	Bennett & Sangster (1981b)
<i>Sorghastrum nutans</i>	Endodermis (ITW, aggregates)	Sangster (1978a)
<i>Sorghum bicolor</i>	Endodermis and stele	Parry & Kelso (1975)
<i>Sorghum bicolor</i> .	Endodermis (ITW, aggregates)	Hodson & Sangster (1989b); Sangster & Parry (1976a, b, c); Ponnaiya (1960); Parry & Kelso (1975)
<i>Sorghum bicolor</i> (aerial roots)	Endodermis (ITW)	Sangster & Parry (1976c)
<i>Triticum aestivum</i>	Endodermis and stele	Hodson & Sangster (1989a)
<i>Triticum aestivum</i> (seminal roots)	Endodermis (mostly ITW)	Bennett (1982)
<i>Triticum aestivum</i> cv. Capelle Desprez (adventitious roots)	No silica detected	Bennett (1982)
<i>Triticum aestivum</i> cv. Little Joss and Hustler	Endodermis (ITW)	Bennett (1982)
<i>Zea mays</i> (adventitious roots)	No silicified root tissue	Bennett & Sangster (1981b)

development typified by modifications to the ITW by the deposition of suberin. Further additions to the cell wall of compounds such as lignin represent the tertiary developmental stage.

Various potential roles have been assigned to the endodermis. The most renowned is that the endodermis acts as a "sorting station" for ion uptake. Due to the Casparian strip and modifications of the ITW, the endodermis prevents any water (and dissolved solutes) entering the stele without first passing across its membrane (see chapter 5), thus allowing for selectivity of substances entering the stele.

An alternative, but not mutually exclusive, function is that the endodermis prevents solute leakage from the stele where the concentration is high (especially in the conducting vessels) to the cortical region (Flowers & Yeo, 1992). Another possible role of the endodermis is to protect the stele from desiccation during water stress (Clarkson & Sanderson, 1971).

Work by Maiti *et al.* (1984) suggested that resistance to *Striga asiatica* parasitization in sorghum cultivars may be related to endodermal thickening, and silica deposition was detected in some resistant cultivars. Therefore, it is possible that the endodermis is a pathogen barrier, preventing entry into the stele and the main conducting vessels of the plant.

### Deposition Mechanism.

In diatoms, silica valve formation occurs within the silica deposition vesicle (SDV), delimited by a membrane termed the silicalemma (Reimann, Lewin & Volcani, 1966). Robinson & Sullivan (1987) reviewed diatom mineralization and suggested the inner surface of the silicalemma had nucleation sites to cause silica polymerization and control deposition, possibly involving cytoskeletal elements. The deposition mechanism of silica within plants, however, is the subject of much debate and many theories have been suggested (reviewed by Sangster & Parry, 1981). As silica deposits can be either intracellular lumen deposits or intercellular deposits, different mechanisms may exist for different sites. In the following review, deposition within the aerial and root regions is separated,

but similar mechanisms may operate in both systems.

### Aerial Deposition.

One theory is based upon the readiness of silica to polymerize when its concentration exceeds about 100 ppm (Iler, 1955), although this value can be affected by other dissolved ions (Birchall, 1978). Water loss during transpiration leads to an increased silica concentration, sufficient to cause silica polymerization without further stimulation. This theory is supported by the fact large numbers of silica deposits occur in areas of high water loss, such as leaves. Hayward & Parry (1980) stated "As these (barley) floral bracts carry out intensive transpiration high silicification is to be expected". However, low transpiration rates may be sufficient to cause sufficient local increases in silica concentration for deposition to occur (Hayward & Parry, 1975). Evidence that transpiration rate may not be essential for silica deposition has been present by various authors (Kaufman *et al.*, 1969; Sangster *et al.*, 1983). Also, the theory can not adequately explain silica deposition in regions of minimal water loss such as roots.

Lumen (intracellular) silicification is possibly related to premature senescence of the cell leading to alteration of structure and/or permeability of the plasma membrane to silicic acid to allow deposition (Sangster, 1970; Hayward & Parry, 1975; Kaufman *et al.*, 1972). In *Triticum aestivum*, the loss of cellular contents was a constant characteristic during the development of silica cells (Blackman, 1969).

Intercellular silica deposits usually occur on or within the cell wall matrix leading to the possibility that silica can bind to organic molecules in plants and prompt polymerization. The fine structure of silica within oat plants suggested that it was deposited in intimate association with the cell wall (Jones *et al.*, 1963). It is feasible that cellulose fibrils of the cell wall may act as a template for deposition (Perry & Fraser, 1991). The authors additionally suggested that fundamental silica particles were formed within the transpiration stream and transported to the deposition site "to ripen". Silica bodies (30-50 nm diameter) have been observed in deposits (in the

lemma of *Phalaris canariensis*, see also root deposition below), although the authors suggested these could be artifacts (Hodson *et al.*, 1984). Sakai & Thom (1979) proposed that silica localized in the stomatal apparatus of sugar cane may interact with the pectins and hemicellulose present, and in fact silica has been shown to bind to plant pectins (Khalil & Duncan, 1981). Perry, Williams & Fry (1987) presented evidence demonstrating that changes in the secondary cell wall polysaccharide synthesis of the hairs from the lemma of *P. canariensis* was concurrent with changes in the arrangement of silica particles. An extensive study was performed by Perumalla & Heath (1991) on the silica deposition associated with fungal infection (see section 3.1) on bean leaves. Using various inhibitors they concluded deposition required phenolics and/or, perhaps, hydroxyproline-rich glycoproteins in the cell wall and cellular activity. The cytoskeleton also appeared to have a role in deposition. How these results relate to non-pathogenic deposition is unclear.

Silica deposition can occur between the epidermis and the cuticle in some plants (see section 3.1). In this area silica may decrease the transpirational water loss leading to suggestions that the silica may be secreted/excreted into the cell wall or through the wall possibly by pores or ectodesmata (Kaufman *et al.*, 1971; Soni & Parry, 1973).

### Root Deposition.

Numerous studies have shown that silica deposition within the endodermis is related to cell wall development (Sangster & Parry, 1976c; Bennett, 1982; Parry & Soni, 1972; Parry & Kelso, 1977; Parry & Kelso, 1975), although the exact component of wall development is unclear. Parry & Kelso (1977) proposed that the nature of the tertiary wall lamellae initiated silica aggregates and that silicification continued after completion of wall thickening. In *Sorghum bicolor* and *Molinia caerulea* there was no evidence that silica was associated with the cellulose layers but it appeared to follow the thickening of the radial and ITW of the endodermis (Parry & Kelso, 1975). Following further analysis, Sangster & Parry (1976c)



proposed that initial deposits in *Sorghum bicolor* endodermis were composed of individual primary spherical units (PSU), diameter 100nm, of silica and these appeared to be aligned with cellulose microfibrils of the cell wall. The authors concluded that deposition was concurrent with cellulose deposition and correlated directly with the state of root tissue maturation. The mechanism they proposed was that silicic acid is transported in the apoplast (see section 5.1) of the cortex until it reaches the Casparian Strips in the endodermal wall; at this point silica moves into the protoplast, where a build-up occurs and the protoplast is forced to excrete silica *via* the wall synthesis pathway or into the stele. They tentatively hypothesized that transport and excretion of monosilicic acid was under the control of the endodermal protoplast and intracellular forces at the cell wall/plasma membrane interface would determine the nature of the subsequent build-up of the solid phase silica aggregates. This was also suggested for the accumulations which occur in bamboo roots (Bennett & Sangster, 1981a). Earlier studies by Parry & Soni (1972) had suggested that silica around the ITW was attributed to the fact that silica movement into the stele is subjected to the regulatory activity of the endodermis and hence there is a tendency for it to accumulate in this layer. Evidence suggests, however, that this is not the case in *Sorghum bicolor* (Sangster & Parry, 1976a). The diffuse type of silicification seen in temperate cereals contrasts with the aggregate deposition observed in tropical cereals (Bennett, 1982), suggesting that deposition mechanisms may be different. A further alternative is that silica concentrates within endodermal walls because of a general accumulation of nutrients within this cell layer and excess silica is removed by deposition within the walls, and it is thought to be a positive and controlled process.

In *Molinia caerulea* silica deposition occurs in the intercellular spaces (ICS) of the cortex layer adjacent to the endodermis (Montgomery & Parry, 1979) prior to endodermal deposition. Initial deposits were spheres lining the cavities, similar to PSU. The authors suggested an apoplastic route for silicic acid across the cortex to the endodermis, water moves

freely into the endodermis and thus the concentration of silica increases on the outside of the cell walls in the cortex, and is deposited. Further, silica distribution in the tribes of the family Poaceae may be determined largely by the phylogenetic status of the genus rather than root morphology (Sangster, 1978b).

Aerial roots also have silica deposits on the ITW of the endodermis (Sangster & Parry, 1976c). The most likely route for silica into aerial roots would be *via* the central vascular system, then "leaking-out" *via* symplastic routes to the endodermis. Thus silica deposition in subterranean, as well as aerial, roots may be as a result of leakage from the stele out towards the cortex being prevented at the ITW (e.g. Parry & Kelso, 1977; Sangster & Parry, 1981; Hodson & Sangster, 1989c; Sangster, 1983).

Sangster & Hodson (1992) reviewed silica deposition in roots, and suggested that the endodermal protoplast is able to mobilize soluble silica. Two mechanisms of accumulation were proposed: a passive model, where accumulation is due to evaporation produced by shoot transpirational water loss, and polymerization occurs due to the cellular environment; and a metabolic mechanism involving specific silica pumps on cell surfaces. The latter is proposed as the most appropriate for roots. Alternatively specific endodermal silicification may be due to endodermal function.

A question which remains unanswered is whether the silica deposits are a necessary enhancement to plant function or an unavoidable consequence of certain cell wall components being present.

### Silica Pools.

It is thought that once soluble silica has converted to deposited silica it is not possible to resolublize it within the plant. Experimental evidence supporting this was presented by Samuels *et al.*, (1991).

The quantity of silica within the plant depends on various factors such as silica concentration supplied but also whether the plant is an active accumulator, a passive accumulator or an excluder of silica (see table 5.1, chapter 5). Deposited silica represents the largest pool of silica, with soluble

silica levels being very low. Fox *et al.* (1969) measured soluble and deposited silica levels in the shoots of sugar cane. Soluble silica was extracted using trichloroacetic acid (TCA). Typically soluble silica was about 17 ppm fresh weight, whereas total silica ranged from 6780-176 ppm dry weight. Soluble silica was highest in the least mature tissue and total silica was highest in recently mature tissue. Hodson & Sangster (1989c) detected presumably soluble silica within the root of *Triticum aestivum* using freeze-substitution, and X-ray microanalysis with transmission electron microscopy. Very little work appears to have been directed towards the measurement of silica pools within higher plants. In diatoms the soluble silica pool can be considerable, Azam, Hemmingsen & Volcani (unpublished data reported in Hemmingsen, 1971) suggested that in *Navicula pelliculosa* and *Nitzschia alba* this pool could be 1-2 mM and, Sullivan (1976), suggested that in *Navicula pelliculosa* free silicic acid levels could reach 5 mM.

The aim of the present investigation was to measure the time course of endodermal silicification in the plants over a ten day growth period in silica nutrient solution. Further to this, was to use microscopical and/or chemical methods to observe silica, either deposited or soluble, in young wheat seedlings within 24 hours after exposure to a silica nutrient solution in order that short-time course experiments could be conducted (see chapter 5). A further aim was to analyze the change in size of the silica pools (soluble and deposited) in the roots and shoots of the plants over the short growth period of ten days and then relate the quantity of deposited silica in the root to the endodermal silicification.

## 4.2. Materials & Methods.

### 4.2.1. Processing for Light Microscopy.

Plants were grown as previously described in section 3.2.1. Si+ solution contained 0.5 mM added silica. After the required growth period in nutrient solution the initial seminal roots were harvested. Sections (1 cm in length) were taken along the length of the root and the length was recorded. The sections were then processed for light microscopy as follows:

Fixed in FAA (formalin: acetic acid: 50% ethanol, 90:5:5) for 30 min..

Dehydrated in 70% ethanol (30 min.), 80% ethanol (30 min.), 90% ethanol (30 min.), 100% ethanol (30 min.), ligroin (3 x 30 min.).

Infiltrated with paraffin wax (melting point 56°C) for 2 x 30 min. in a waxing oven.

Embedded in plastic moulds containing paraffin wax, five specimens per mould.

The wax blocks were then sectioned on an AO Spencer 820 Rotary Microtome at 15µm thick, floated on warm water and mounted on clean microscope slides. These were then gently dried on a hot plate.

For ashed sections the technique of microincineration was used (Jensen, 1962). The sections on slides were heated in a Gallenkamp muffle furnace at 400°C for 4 hours.

### 4.2.2. Staining Techniques for Silica

Three stains were used for light microscopy to detect silica in root sections, these were crystal violet lactone (CVL, 0.1% w/v in xylene [Dayanandan, Kaufman & Franklin 1983]), methyl red (MR, 1% w/v in absolute ethanol, then diluted 2:25 with xylene [Dayanandan *et al.*, 1983]) and toluidine blue (TB, 0.25% w/v aqueous solution [Curran, 1953; Sangster, 1970]).

Cleared sections (20 min. in xylene) were stained using either CVL or MR, for up to 20 min., then rinsed with xylene and either viewed directly or mounted in Canada balsam or DPX. Ashed sections were also stained in a similar manner. When using TB the cleared sections were rehydrated (through 100% ethanol, 70% ethanol and distilled water) prior to staining. The sections were stained for 20 min., rinsed in distilled water and dehydrated (in 100 % ethanol followed by xylene) and mounted in Canada balsam. Ashed sections were also stained.

#### 4.2.3. Whole Section Staining.

Root sections were also stained with safranin (0.1 % in 100 % ethanol). They were cleared in xylene for 20 min. and then transferred to 100 % ethanol for 5 min. After this time, they were placed in the safranin stain and left until they appeared red (about 10 min.). The sections were washed in 100 % ethanol followed by a wash in xylene and mounted in Canada balsam. This technique was used to determine the number of endodermal cells present in each section.

Stained root sections were observed using a Vickers MSC light microscope or a Zeiss standard epi-fluorescence light microscope. All black & white photography used Ilford HP5 Plus film.

The percentage silicification of the endodermis was calculated by counting the number of endodermal cells stained when using a silica specific stain and dividing by the total number of endodermal cells determined from safranin stained sections.

#### 4.2.4. Fluorescence Microscopy.

A specific fluorescent stain for silica was investigated for its use on plant samples. Fluorescein isothiocyanate (Isomer I [FITC], obtained from Sigma) was covalently linked to 3-aminopropyltriethoxysilane ([APS] obtained from Aldrich, Gillingham, Dorset), after Van Blaaderen (1992). 78.5 µl of APS and 2.697 ml 100 % absolute II ethanol was mixed then 0.01 g FITC was added. This was left in a covered glass vial stirring for

24 hours. This was termed FITC-APS solution.

Various samples were stained using the FITC-APS solution. Macrohairs from the inflorescence bracts of *Phalaris canariensis* grass (a gift from Dr. C. O'Neill, Imperial Cancer Research Fund, London) are known to contain silica (Hodson, Sangster & Parry, 1984). Both native and acid-treated (to remove organic matter) hairs were investigated. Cleared wax embedded root sections from the basal area (i.e. close to the caryopsis) of wheat grown in Si<sup>+</sup> or Si<sup>-</sup> solution for 10 days were also investigated. Two types of Spurr resin embedded root samples were stained. One type was wheat root samples from section 4.2.5. The other samples were freeze-substituted *Sorghum bicolor* roots (donated by M. J. Hodson) which had been previously shown to contain silica within the endodermal cell wall (Hodson & Sangster, 1993).

Staining of *Phalaris* hairs was performed by placing a small sample (typically 0.01g) in a glass vial and adding sufficient FITC-APS solution to cover the hairs. Once the cap of the vial had been replaced, the vial was covered completely in aluminium foil and stirred for up to 2 h.. Following this, the sample was washed 4 times in 1.5 ml 100% ethanol and placed on a glass microscope slide. "Citifluor" in glycerol was used as a mountant and the coverslip was sealed with clear nail varnish. A Zeiss standard epi-fluorescence microscope, with an FITC filter set was used to view the sample and black & white photography used Ilford HP5 Plus film.

For staining wax embedded root sections with FITC-APS solution, the slide was firstly dewaxed in xylene for 15 min., and then washed in 100 % ethanol for 10 min. and stained as described below. The resin embedded sections were stained directly in the following manner. A petri-dish was prepared by lining it with "cling-film". On top of this was placed filter paper soaked in ethanol to maintain a high ethanol concentration in the dish and therefore reduce the amount of evaporation of ethanol from the stain. The slides were then placed in the petri-dish and enough stain was added to completely cover the slide. The petri-dish was covered with foil. The sections were stained for 110 min.. After staining the FITC-APS solution

was gently poured off the slide and the slide placed in 100 % ethanol for 10 min. to wash. Then the slides were removed from the ethanol, drained, and mounted and viewed as before.

#### The Effect of FITC on Different Samples.

The stain prepared was 5 mg FITC dissolved in 2.5 ml 100 % absolute II ethanol, without the addition of APS. This was to determine whether FITC alone would stain the samples.

#### An Investigation of the Autofluorescence.

The degree of autofluorescence in the wheat root sections was investigated. The wax sections were dewaxed in xylene for 15 min., washed in 100 % ethanol for 10 min., and then mounted either in citifluor as described previously, or mounted in ethanol or water with a sealed coverslip. The resin embedded sections were either mounted in citifluor or were left uncovered. Unstained native *Phalaris* hairs were also investigated. The hairs were placed on a glass slide and a coverslip was added and sealed.

#### 4.2.5. TEM Investigations.

Plants were grown in Si<sup>-</sup> solution for 5 days and then transferred to Si<sup>+</sup> solution (culture details as in section 3.2.1, Si<sup>+</sup> solution contained 0.5 mM added silica). The roots were harvested 3, 6, 12 and 24 hours later. The section of the root taken was 30 mm from the caryopsis of the plant. The sections were fixed overnight in 3% glutaraldehyde in sodium cacodylate buffer (0.05M, pH 7.0) at 4°C. They were then washed in buffer (6 x 90 min.) and remained in buffer overnight at 4°C. This was followed by post-fixation in osmium tetroxide (2% aqueous solution) for 6 hours. Dehydration of the specimens was performed through an ethanol series, 20 % ethanol (30 min.), 50 % ethanol (30 min.) and transferred to 70 % ethanol, remaining in this overnight. The specimens were then passed through 90 % ethanol (30 min.), 100 % (absolute I) ethanol (4 x 15 min.)

and finally 100 % (absolute II) ethanol, (6 x 20 min.). Resin infiltration was carried out through an ethanol/Spurr resin mix over 4 days (ethanol:resin, 1:3, 1:1, 3:1). The samples remained in pure resin for 3 days, with the resin being changed twice a day. Polymerization was carried out at 70°C for 8 hours.

The blocks were sectioned on a Reichert-Jung ultramicrotome at 100 nm and floated on to ultra pure water. Sections were picked up on copper grids and stained with uranyl acetate (2% methanol solution for 5 min.) and lead citrate for 15 min. (Reynolds, 1963). They were then viewed and photographed using a JEOL 1200EXII TEM at 80 kV.

#### 4.2.6. X-ray Microanalysis Studies.

X-ray microanalysis was performed at three different locations in Canada. The SEM study was carried out at the Carleton University in Ottawa. The facility at Mount Sinai Hospital in Toronto was used for the TEM study and cryo-SEM was performed at the McMaster University in Hamilton.

Thick sections (about 200 nm, purple interference colour) were cut from the blocks prepared in section 4.2.5 to be examined using X-ray microanalysis. For analysis using scanning electron microscopy the samples were allowed to dry either directly on the aluminum stub or dried on to a plastic cover-slip which was then fastened to the stub using double-sided tape. The specimens were then coated with carbon and analyzed using a SEM with X-ray microanalysis facility (20 kV operating voltage, 65 mm working distance, 150 secs. live time; see Hodson & Sangster, 1993 for further details). Samples for TEM analysis were mounted on formvar coated copper grids and analyzed using the TEM (mag. 17k, 100 secs. live time, see Hodson & Sangster, 1993 for further details).

Frozen samples were also analyzed. Wheat plants (note different cultivar, Wheaton) were grown as previously described (section 3.2.1) for 24 h. with an inhibitor, sodium orthovanadate (obtained from Aldrich) being added to the pots at 1 mM. The plants were transported to the electron



microscopy facility. The roots were removed and the basal area frozen (in liquid nitrogen slush), fractured and etched prior to analysis using the cryo-SEM with X-ray microanalysis facility (see Hodson & Sangster, 1989c for further details).

#### 4.2.7. Chemical analysis for silica.

##### 4.2.7a. Sample Preparation.

##### **Total Silica Determination.**

The acid digestion method described here is based upon the method of Nayer, Misra & Patnaik (1975).

The plants were harvested after up to 10 days growth in water culture (as described in section 3.2.1) and the roots and shoots were dried separately overnight (at least) in an oven at 60°C. 10 mg of dried plant material was acid digested in 5 ml Pyrex conical flasks using 0.5 ml conc. nitric acid. This was boiled on a Gallenkamp sand-bath (at about 200°C) until the brown fumes ceased, and the volume remaining was about 0.2 ml. Stainless-steel crucibles (capacity, 25 ml) containing 0.4 g anhydrous sodium carbonate (AnalaR quality from BDH) in 0.5 ml ultra-pure water were then prepared. The solution in the flask was transferred to the crucible, which was covered with a nickel lid to prevent loss of solution. The lid was wrapped in aluminium foil to stop the acid damaging the lid. The flask was then washed out with 1 ml ultra-pure water, the washings being added to the crucible. The solution in the crucible was boiled (30-60 secs) to depolymerize deposited silica. When cool, the contents of the crucible were transferred to a 10 ml plastic measuring cylinder and the volume was made up to 2.4 ml with ultra-pure water washings from the crucible. This was transferred to a plastic test-tube. One ml of 6 M HCl was added to the plastic test-tube to reduce the pH, and the sample was whirl-mixed to remove the carbon dioxide bubbles which had formed. This solution will in future be referred to as the prepared sample.

### **Soluble Silica Determination.**

Fresh plants were used for the analysis of soluble and total silica and the xylem content of silica. For analysis of fresh plant samples the above technique was used except that the quantity of plant material was increased to 100 mg and the quantity of nitric acid increased to 1 ml. This was then boiled on the sand-bath until the brown fumes ceased and the quantity of acid reduced to 0.2 ml.

When soluble silica determination was required the depolymerization step (boiling with sodium carbonate) and the addition of HCl was omitted. The final volume of 3.4 ml was made up with ultra-pure water. This was then a prepared sample.

### **Determining Silica Concentration in the Xylem Sap.**

Xylem silica content was determined by collecting xylem exudate 90 min. after the removal of shoots. The shoots were removed 10 mm from the base of the plant and the cut end was covered with an eppendorf tube, which had the base removed, to prevent evaporation from the exudate. The droplets which formed were collected with a gel-loading tip on a Gilson automatic pipette. The xylem exudate from 4 plants was bulked together for each sample. There were no further preparative steps on these samples.

#### **4.2.7b. Determination of Soluble Silica in the Prepared Samples.**

This spectrophotometric determination of silica is based on the method of Fox *et al.* (1969) and was used for all of the above prepared samples. Silica standards were prepared from a 1.25 mM Si solution, freshly prepared from sodium metasilicate  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ .

Ultra-pure water was used throughout the silica determination and new plastic test-tubes were used for each determination. The solution was whirl-mixed after each addition of reagent. To 6000  $\mu\text{l}$  of water, 400  $\mu\text{l}$  of prepared sample (excluding xylem exudate samples) was added. The ammonium molybdate reagent (see appendix II) was then added (200  $\mu\text{l}$ ) and the yellow colour was allowed to develop for 30 min., for the formation

of silicomolybdate and phosphomolybdate complexes. The interference by the phosphomolybdate complex was removed by adding 600  $\mu$ l 10% oxalic acid and left for 2 min.. The reducing solution (see appendix II) was added and the blue colour allowed to develop for 30 min.. The final volume was then made up to 10 ml using 2600  $\mu$ l of water. The samples were then read at 660 nm using a Perkin Elmer lambda 3B spectrophotometer using 1 ml plastic cuvettes (standard curve presented in appendix IV).

#### 4.2.7c. Determination of Soluble Silica in Xylem Samples.

The same method as above was used except that the final analysis volume was 1000  $\mu$ l instead of 10 ml, with all the volumes in the above protocol being reduced appropriately. (600  $\mu$ l water, 5  $\mu$ l of xylem exudate, 20  $\mu$ l molybdate reagent, left for 30 min., 60  $\mu$ l 10 % oxalic acid, left for 2 min., 20  $\mu$ l reducing solution, 295  $\mu$ l water and left for 30 min.). The samples were then read at 660 nm as above.

### 4.3. Results.

#### 4.3.1. Staining Techniques.

##### **Crystal Violet Lactone.**

There was no staining observed using this dye on either cleared wax embedded root sections or on ashed sections, even after the staining time was increased to 20 minutes.

##### **Methyl Red.**

This stained the whole root section when used on cleared wax embedded sections from plants grown in either Si<sup>+</sup> or Si<sup>-</sup> solution. It was clearly not specifically staining silica. When used on ashed sections endodermal cells of plants grown in Si<sup>+</sup> were stained a light pink. This staining was not considered to be strong enough to be of any practical use. Also the staining faded when a either Canada Balsam or DPX was added as a mountant.

##### **Toluidine Blue.**

This also stained the whole root section when used on cleared wax embedded sections. The whole section was stained purple/blue. The endodermis appeared a light blue as did the stele. Nuclei appeared purple. When used on ashed sections toluidine blue only stained certain endodermal cells of roots grown in Si<sup>+</sup> solution and did not stain the Si<sup>-</sup> grown plant root sections. The staining was strong and permanent initially when Canada balsam was added, although it faded over an extended time. This stain was used to determine percentage of endodermal cells silicified in ashed root sections. The total number of endodermal cells in the root section was determined by staining with safranin.

*Percentage*

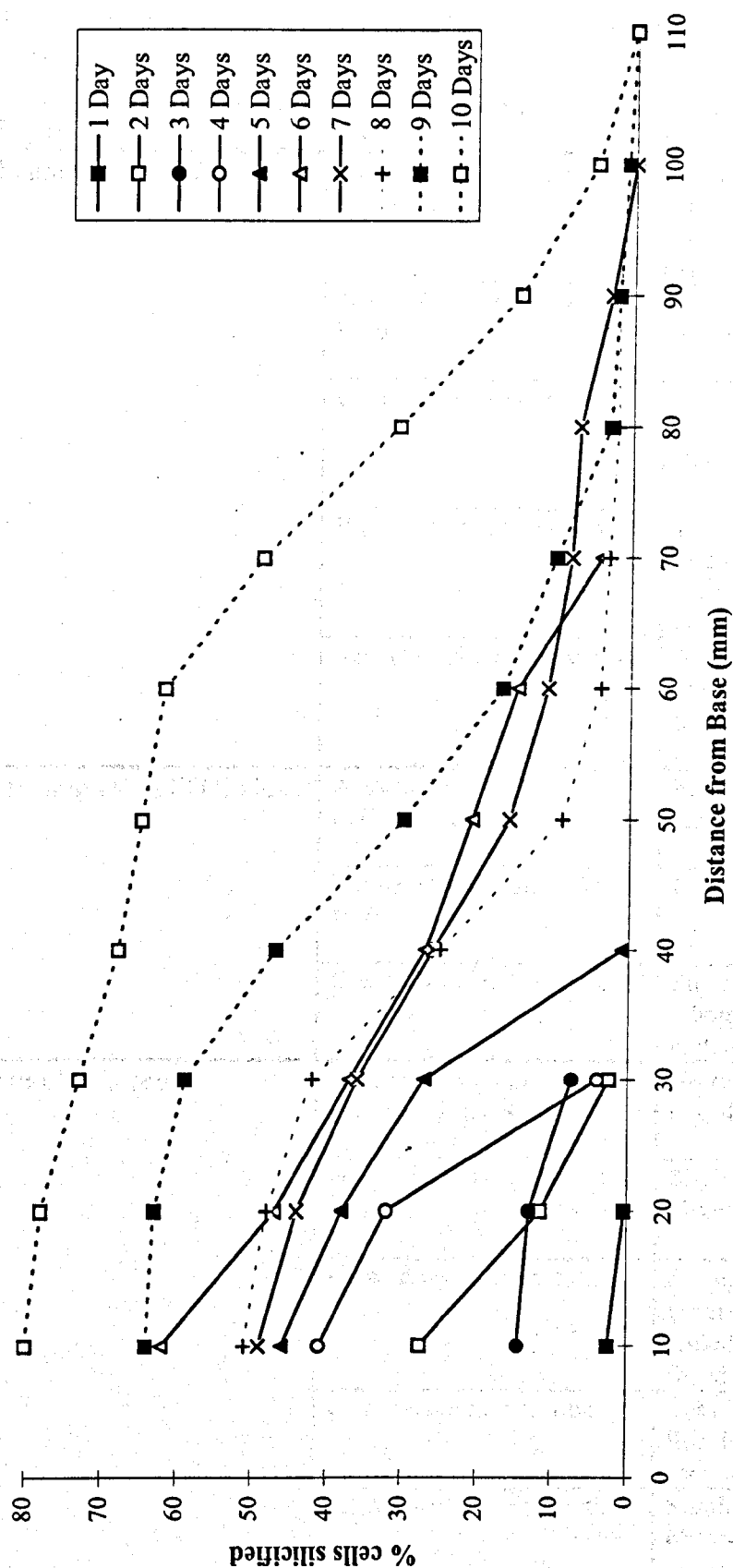
$$\text{endodermal cells} = \frac{\text{Number of cells stained with toluidine blue}}{\text{Number of endodermal cells within section}} \times 100$$

*silicified*

Figure 4.1 shows the percentage of endodermal cells silicified in roots grown for up to ten days in Si<sup>+</sup> solution (the roots of plants grown in Si<sup>-</sup> solution did not show any silicification of the endodermal cells detectable with toluidine blue staining). Silicification is greatest in the basal area of the root (i.e. the area of the root closest to the caryopsis) and decreases down the root to the apex. The apex of the root shows the least amount of silicification, usually showing no silicification of endodermal cells (where there is differentiation). The percentage of endodermal cells silicified increases with the length of time the plant grows in the silica nutrient solution. An interesting point on the graph is 40 mm down the root, where there appears to be no increase in the percentage of cells silicified for three days (day 6-8). Then on day nine the percentage silicified increases and continues to increase on day ten. Roots grown for ten days showed a much greater percentage of endodermal cells silicified than those roots grown for nine days. This difference appeared to be greater than the difference between any other consecutive days. After 24 hours there was some silicification of endodermal cells (detectable with toluidine blue), mostly in the basal area (5.63% stained). After ten days in Si<sup>+</sup> nutrient solution the same area of the root showed that 80% of the endodermal cells have silica deposited in the inner tangential wall.

#### 4.3.2. Fluorescence Studies.

An investigation was carried out using a silica coupling agent 3-aminopropyltriethoxysilane (APS) covalently linked to FITC. It was hoped this would specifically stain silica deposited in root sections, preferably after the plants had only been exposed to silica solution for 24 hours, and thus be a more sensitive technique than using the toluidine blue stain. The results are summarized in table 4.2. FITC-APS stained the acid-extracted silicified hairs of *P. canariensis*. A two hour staining time gave strong fluorescent staining (fig. 4.2a and b) although slight staining occurred within 10 min. There was reduced staining of native hairs. The autofluorescence of wax embedded root sections from plants grown in silica



**Figure 4.1.** The percentage of endodermal cells silicified, as determined by staining with toluidine blue, in the roots of wheat plants grown for up to 10 days in 0.5 mM Si nutrient solution. Base is defined as the root area closest to the caryopsis, as opposed to the apical region. The data are mean averages and the number of replicates varies between 7-32.

**Table 4.2.** A summary of the results from the fluorescence investigation using FITC-APS solution.

Experiment	Sample	Comments
Staining using FITC-APS	Acid-treated <i>Phalaris canariensis</i> hairs	Staining occurred within 10 min. and intensity increased with increased staining time (up to 2h., fig. 4.2a, b).
	Native <i>Phalaris canariensis</i> hairs	The staining observed was similar to the above.
	Cleared wax root sections	Strong fluorescence in whole section, especially in the endodermis of both Si + and Si - plants (fig. 4.2c).
	Resin wheat root section	Fluorescence observed in endodermis and pericycle cells and various cortical cells.
	Resin <i>Sorghum</i> root section	Non specific fluorescence observed in endodermis and pericycle cells and various cortical cells.
Staining using FITC alone	Acid-treated <i>Phalaris canariensis</i> hairs	Faint fluorescence observed (fig. 4.2d).
	Native <i>Phalaris canariensis</i> hairs	Faint fluorescence observed.
	Cleared wax root sections	Strong fluorescence of whole section, especially the endodermis (fig. 4.2e).
Autofluorescence	Acid-treated <i>Phalaris canariensis</i> hairs	No fluorescence observed (fig. 4.2f).
	Native <i>Phalaris canariensis</i> hairs	Slight fluorescence observed.
	Cleared wax root sections	Strong fluorescence observed, especially in the endodermis. Fluorescence faded slightly over 30 min..
	Resin wheat root section	Less fluorescence observed than in cleared wax sections.
	Resin <i>Sorghum</i> root section	Similar fluorescence to that observed in resin wheat sections.

**Figure 4.2.** Light micrographs of silicified plant material stained using either FITC-APS, FITC alone or left unstained.

A-B) Fluorescence (A) and phase light (B) micrographs of *P. canariensis* hairs (acid-extracted) stained with FITC-APS for 2 h. (x 100).

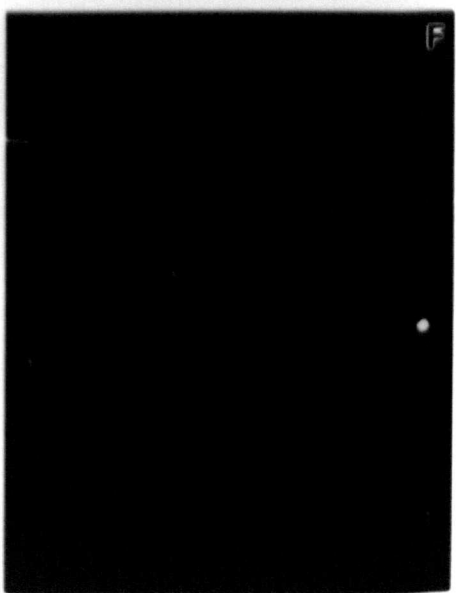
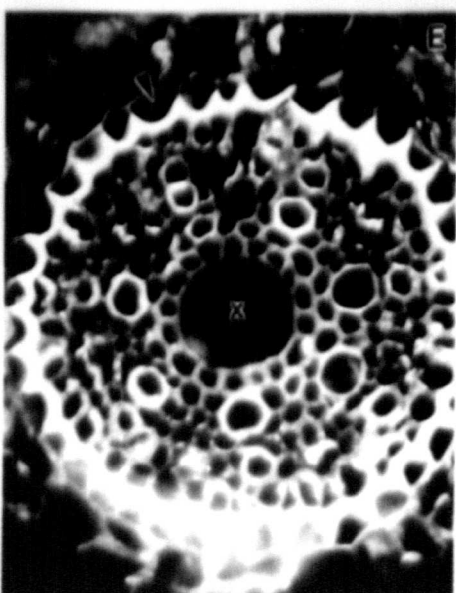
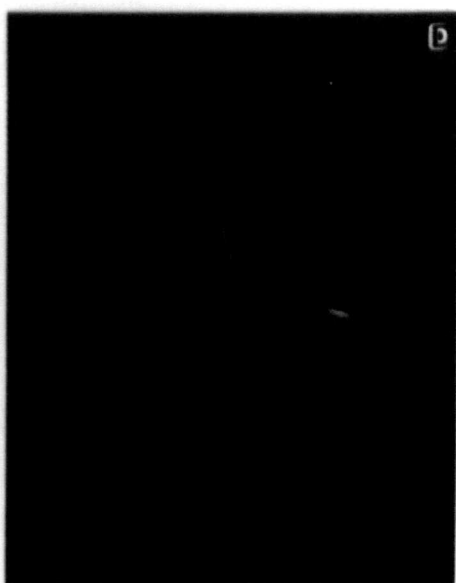
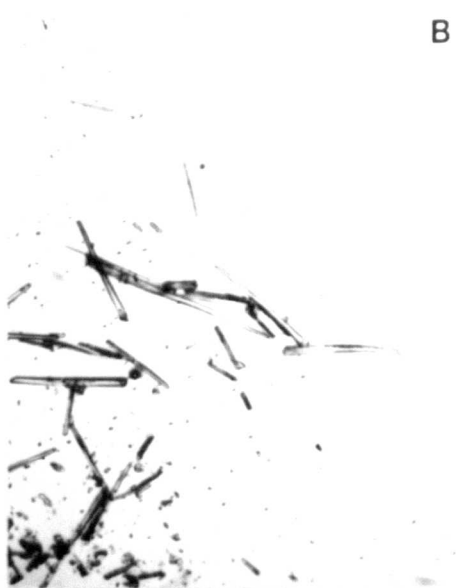
C) Si<sup>+</sup> plant basal root section stained for 40 min. with FITC-APS, mounted in ethanol only (x 100).

D-E) Fluorescence micrographs of silica containing samples stained with FITC (dissolved in ethanol) for 2 h. D) Native *P. canariensis* hairs

showing slight fluorescence (x 100). E) The stele region of a Si<sup>+</sup> plant root section, clearly showing the amount of fluorescence associated with the endodermis (arrow) and the xylem vessel lumen is indicated by x (x 450).

F) Fluorescence micrographs showing the autofluorescence associated native *P. canariensis* hairs (x 100).





added solution and plants grown in silica minimal solution, was a problem. It was not possible to determine if the FITC-APS was staining any silica within these sections (fig. 4.2c) as fluorescence was seen in these sections when stained with FITC alone (fig. 4.2e). Only slight staining of *P. canariensis* hairs was observed when FITC alone was used (fig. 4.2d). There was no autofluorescence observed in the *P. canariensis* hairs (fig. 4.2f). There was also considerable background staining of the slide itself and precipitation of stain onto the glass slide. When using Spurr resin embedded root sections, which displayed less autofluorescence, the stain did not appear to stain a specific area. When the stain was used on *S. bicolor* root sections, with known silica deposits, there still did not appear to be any specific staining.

#### 4.3.3. TEM Investigations.

Electron micrographs of sections from the basal area of the roots from (six day old) wheat plants are presented in fig. 4.3. From these it can be clearly seen that the inner tangential wall of the endodermal cell wall is already thicker than the neighbouring cell wall of the pericycle (fig. 4.3a and c). The main objective for this study was the observation of possible silica deposits within the cell walls of root section, and therefore the lack of preservation of the ultrastructure of the cytoplasm was of little concern.

It would appear that after only 24 hours in an added silica nutrient solution (0.5mM, Si<sup>+</sup>), electron opaque deposits were already present in cell walls of the endodermis and small xylem vessels (Fig. 4.3b-d), which are not present in the roots of plants which remained in nutrient solution without added silica (Si<sup>-</sup>) (Fig. 4.3a). These deposits may be silica.

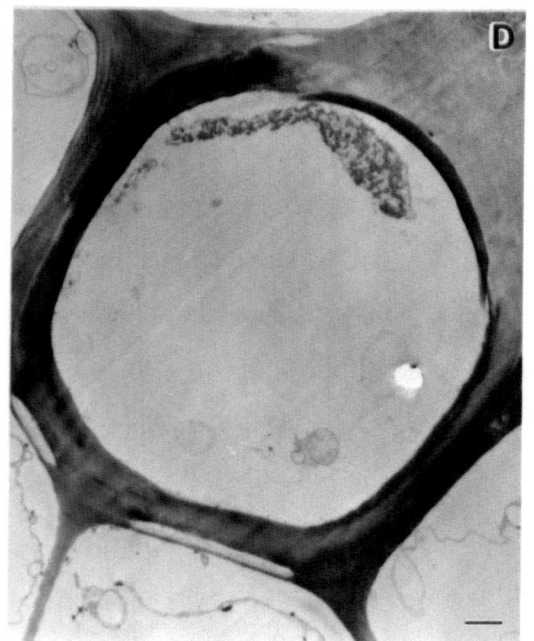
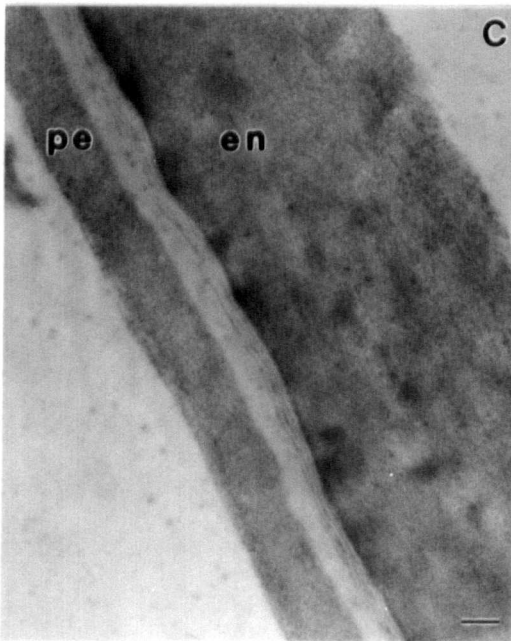
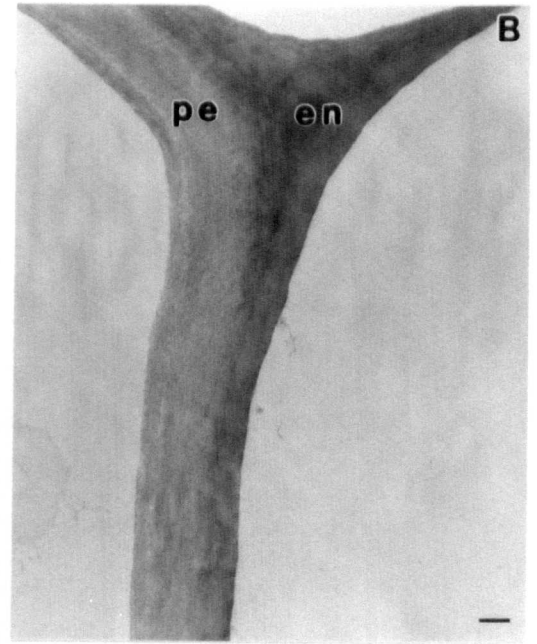
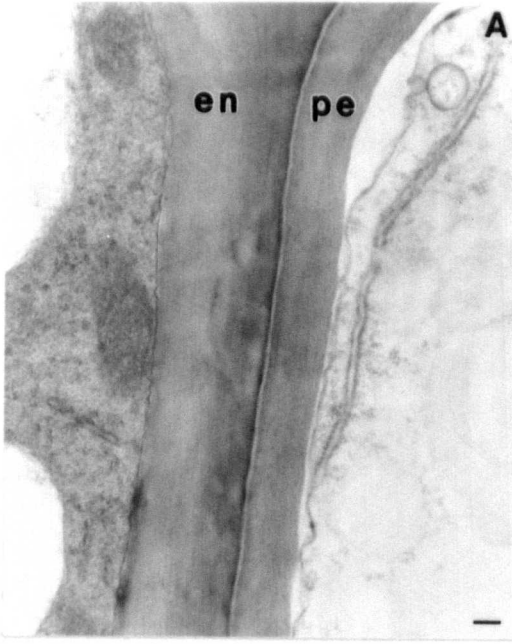
**Figure 4.3.** Transmission electron micrographs of the basal area of the roots (i.e. area closest to caryopsis) of wheat plants grown in Si<sup>-</sup> nutrient solution for 5 days followed by either 24 hours in Si<sup>+</sup> (0.5 mM Si) solution or in Si<sup>-</sup> nutrient solution (en=endodermal wall, pe=pericycle wall)

A) The inner tangential wall of the endodermis and the pericycle wall of plants grown in Si<sup>-</sup> nutrient solution. Bar=200nm.

B) The endodermal/pericycle wall of plants grown in Si<sup>+</sup> nutrient solution. The endodermal wall appears darker than the pericycle wall. Bar=200nm.

C) The endodermal/pericycle wall of plants grown in Si<sup>+</sup> nutrient solution. The endodermal wall shows dark deposits and appears wider than the pericycle wall. Bar=50nm.

D) A metaxylem vessel from a plant grown in Si<sup>+</sup> nutrient solution. There appears to be dark deposits around the walls of the vessel. Bar=1µm.



#### 4.3.4. X-ray Microanalysis Studies.

##### Scanning Electron Microscopy.

Low magnification micrographs of resin embedded root sections were taken using the SEM at Carleton University, Ottawa, Canada (fig. 4.4). There do not appear to be any gross morphological difference between root sections from Si- plants and Si+ plants (compare fig. 4.4a and 4.4c). Higher magnification of the endodermal region of the roots clearly shows the variable thickness of the inner tangential wall (ITW) of endodermal cells in both Si- (fig. 4.4b) and Si+ plants (fig. 4.4d). The root sections photographed here have two large metaxylem vessels present, however it is more typical of this particular cultivar, Beaver, to have one large central vessel as observed during the light microscopy investigation.

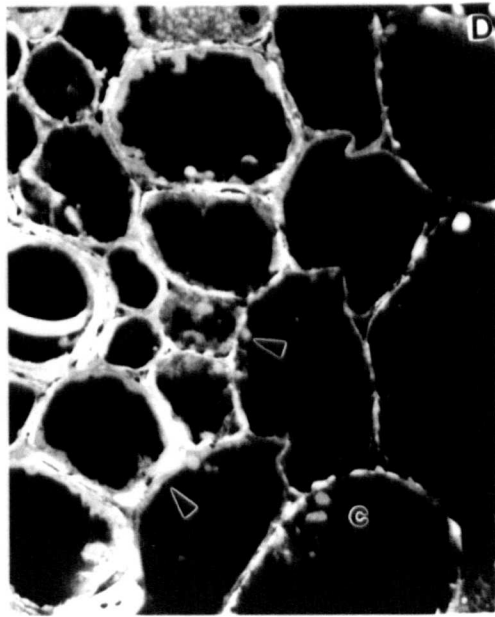
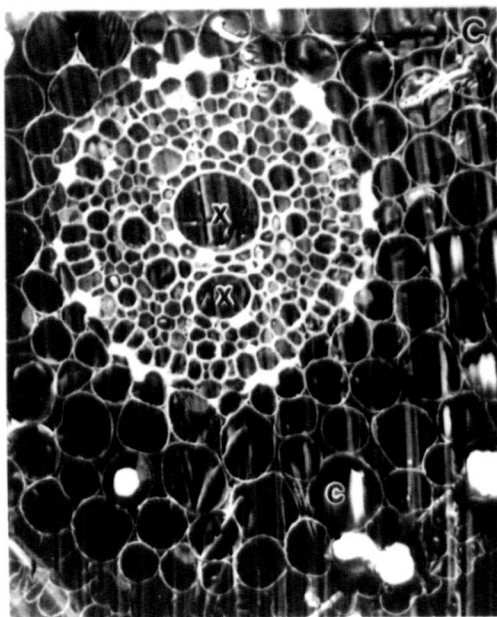
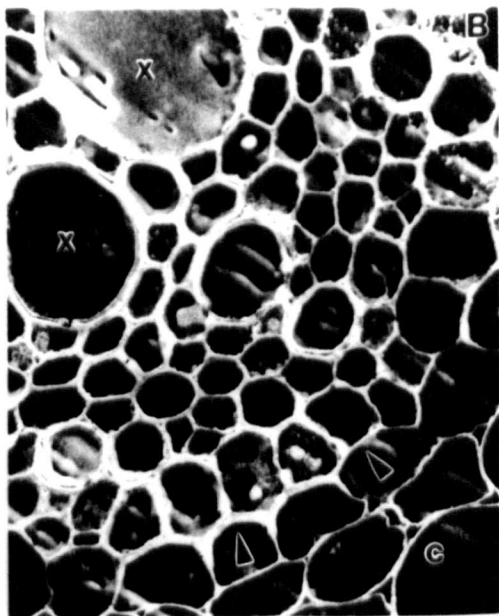
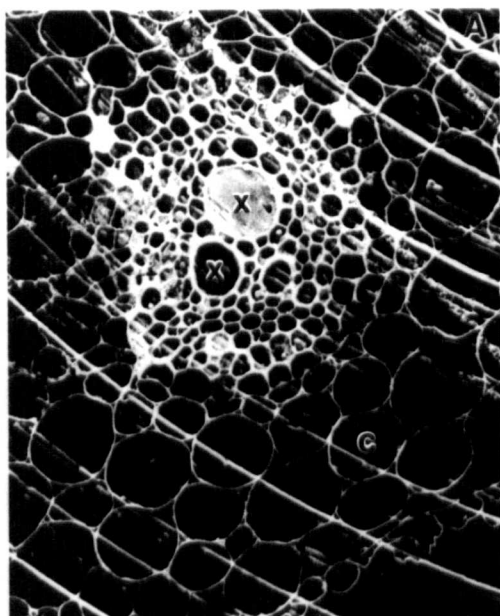
X-ray microanalysis of the ITW of endodermal cells of the basal area of the root yielded very little data in respect to silica deposition. Often the electron beam destroyed the thin tissue of the section and therefore metals from the stub were analyzed with the elements in the section. The X-ray spectra presented (fig. 4.5) were typical of those obtained. The aluminum peak was due to analyzing the aluminum stub, as were the copper peaks. Any silicon peaks detected were very small, often masked by the osmium peak (osmium tetroxide was used as a fixative<sup>1</sup>), as can be seen in fig. 4.5a. Silicon was not detected in all root sections exposed to silica solution, and there did not appear to be an increase in the amount of silicon detected over the increasing exposure to silica solution. Also silica deposition appeared to be patchy, such that silicon was not detected in all the ITW of endodermal cells in a section but was often isolated to a few cell walls. The control plants appeared not to contain silica (fig. 4.5c).

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<sup>1</sup> Osmium tetroxide has been used in previous reports (Sangster & Parry, 1976c), but the level of silica in the present samples was very low and it was not possible to repeat this investigation omitting the osmium tetroxide during the time available in Canada.

**Figure 4.4.** Scanning micrographs of resin embedded basal root (i.e. close to the caryopsis) sections of plants initially exposed to Si<sup>-</sup> nutrient solution followed by exposure to Si<sup>+</sup> (0.5 mM Si) nutrient solution. x=xylem vessel, c=cortical cell.

- A) Sections from a plant not exposed to Si<sup>+</sup> nutrient solution (x 300).
- B) Higher magnification of 4.4A. The endodermal cell wall (arrow) shows variations in thickness (x 900).
- C) Sections from a plant exposed to Si<sup>+</sup> nutrient solution for 3 hours (x 300).
- D) The basal area of root of a plant exposed to Si<sup>+</sup> nutrient solution for 24 hours. The endodermal wall (arrow) displays varying thickness (x 1600).



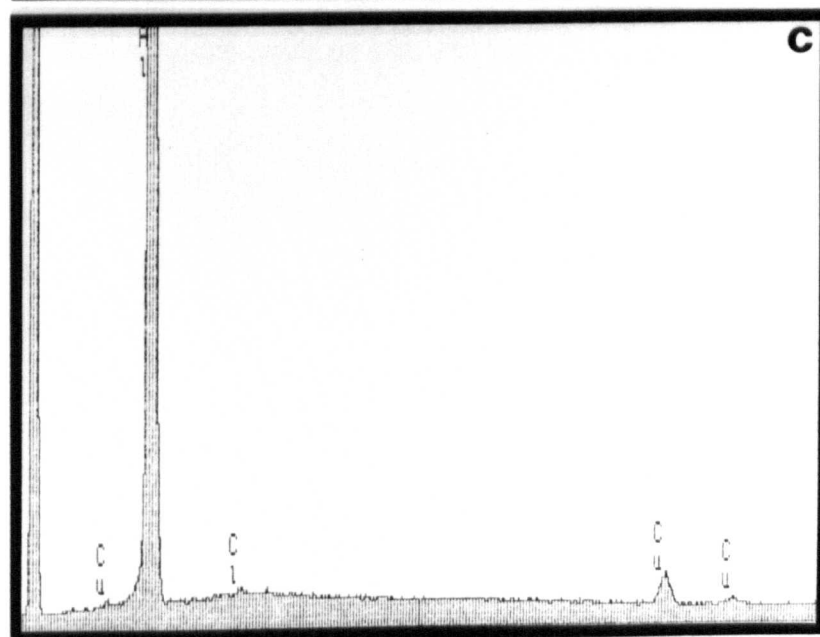
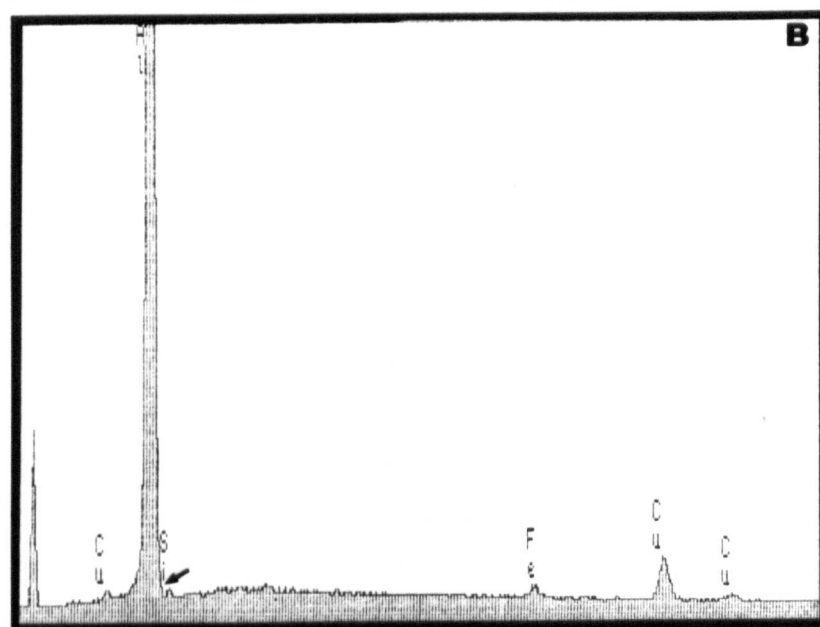
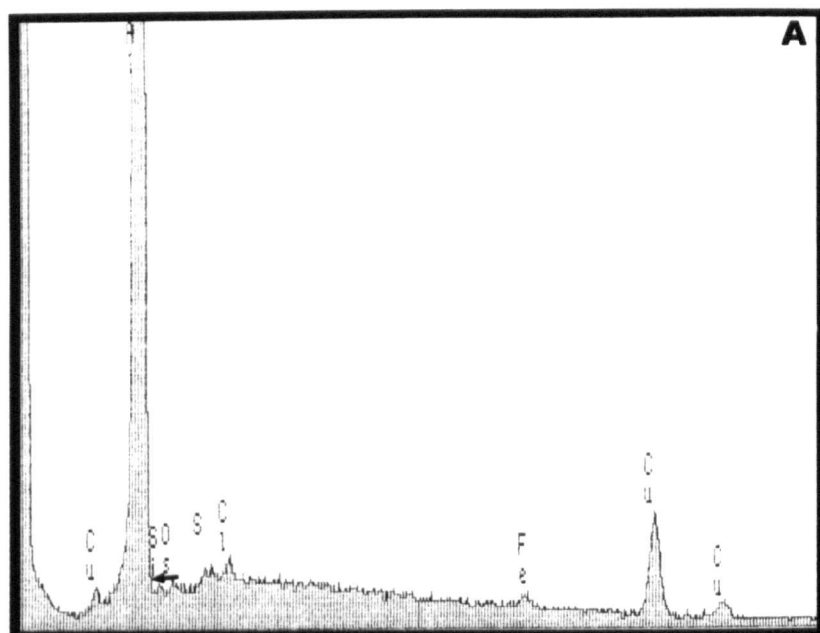
**Figure 4.5.** X-ray microanalysis spectra from root sections embedded in Spurr resin mounted on aluminium stubs, viewed using SEM. The root section were from the basal area (i.e. closest to the caryopsis) of plants grown for 5 days in Si<sup>-</sup> solution and then transferred to Si<sup>+</sup> solution (0.5 mM Si). The times correspond to the length of time the plant remained in Si<sup>+</sup> solution before harvest. Control plants remained in Si<sup>-</sup> solution.

A) Spectrum from the ITW of endodermal cells of a root exposed to Si<sup>+</sup> solution for 12 h.

B) Spectrum from the ITW of endodermal cells of a root exposed to Si<sup>+</sup> solution for 24 h.

C) Spectrum from the ITW of endodermal cells of a root not exposed to Si<sup>+</sup> solution and therefore used as a control.





0 keV 10

### Cryo-SEM.

This technique is capable of measuring soluble silicon as well as deposited silicon, as soluble silicon is washed out when processing for conventional microscopy. The ITW of the endodermal cells in the basal area of the root were analyzed. Again little silicon was detectable in plants exposed to silica solution for 24 h. (fig. 4.6a), and therefore using an inhibitor, sodium orthovanadate, to prevent silica deposition had very little effect on the quantity of silica detected (fig. 4.6b).

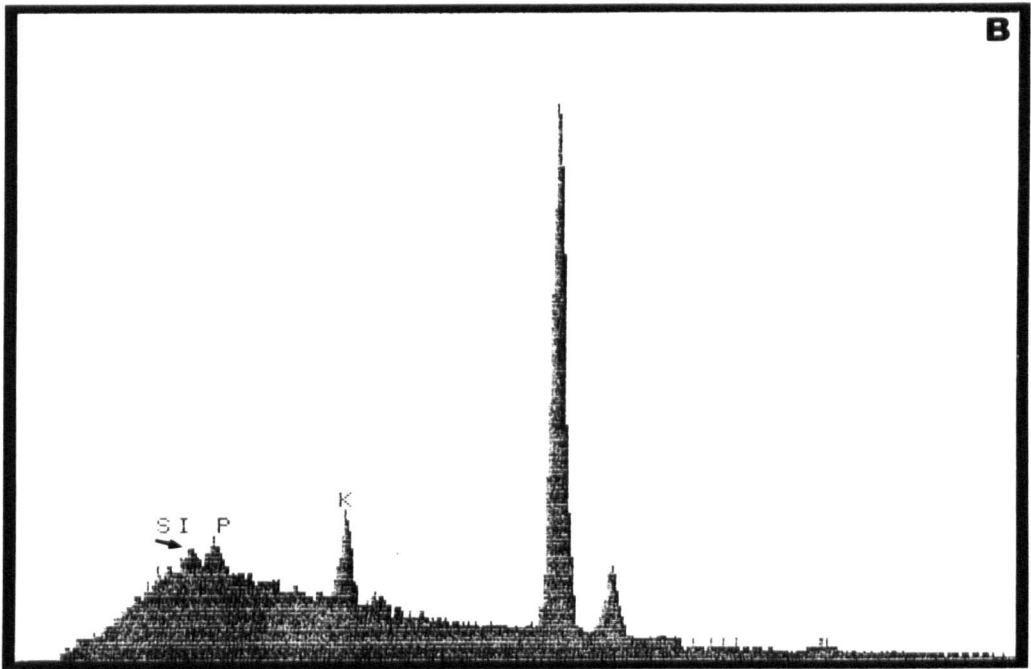
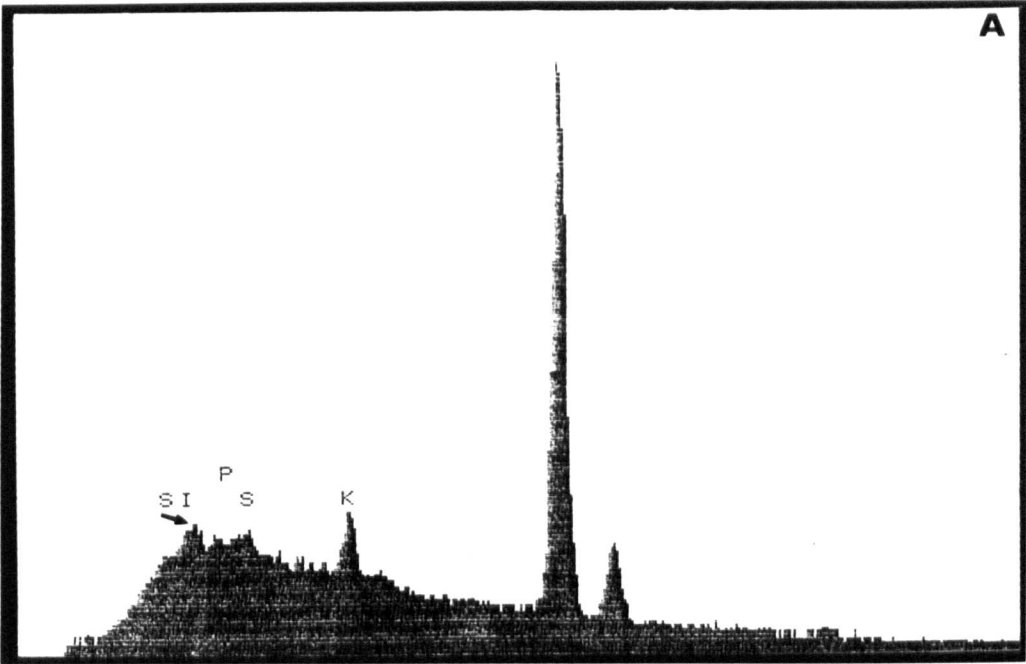
### Transmission Electron Microscopy.

Large copper peaks were detected due to the samples being on formvar coated copper EM grids. Again osmium peaks obscured small silicon peaks. The Si- (control) plants did show a small silicon peak (fig. 4.7a). In some cases this peak was greater than plants exposed briefly to silica solution (fig. 4.7b). A small Si peak was detected in the radial walls of the endodermal cells after the plant had been exposed to Si for 12 h. (fig. 4.7c). A stronger silicon peak was observed in the ITW after 24 h. exposure to silica solution (fig. 4.7d). However, as noted above, this result was not consistent, and not all the ITW of the endodermal cells contained detectable quantities of silica (fig. 4.7f). An interesting area was an electron opaque "spot" within the ITW of an endodermal cell of plants exposed to silica for 24 h. (fig. 4.7e). This spot gave a very strong silicon peak although the surrounding wall gave a low background reading for silicon, see figure 4.7f (a technical problem with the lens of the microscope prevented any photography).

**Figure 4.6.** X-ray microanalysis spectra from root sections which had been frozen. The root section were from the basal area (i.e. close to the caryopsis) of plants grown for 5 days in Si- nutrient solution and then transferred to either Si+ nutrient solution or Si+ solution containing sodium orthovanadate (1 mM).

A) Spectrum from ITW of the endodermal cells of roots from plants exposed to Si+ solution for 24 h.

B) Spectrum from ITW of the endodermal cells of roots from plants exposed to Si+ solution containing sodium orthovanadate for 24 h.



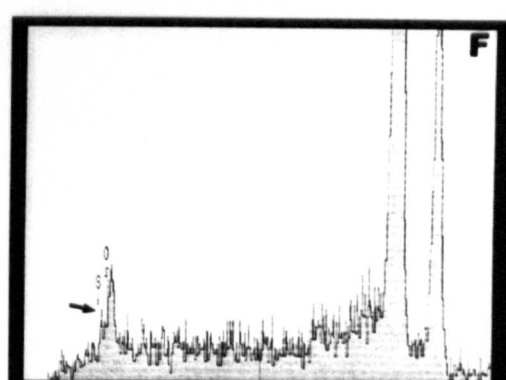
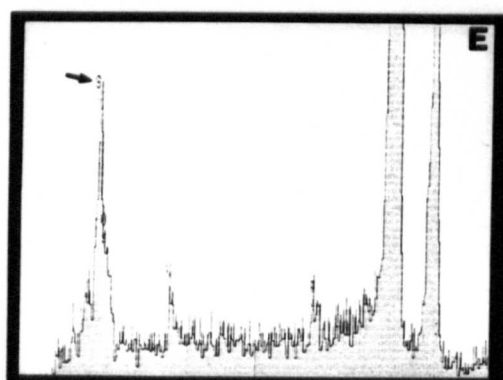
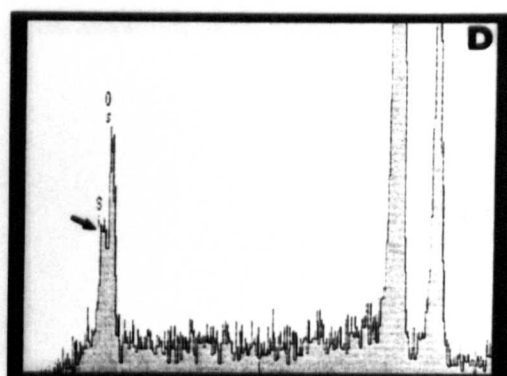
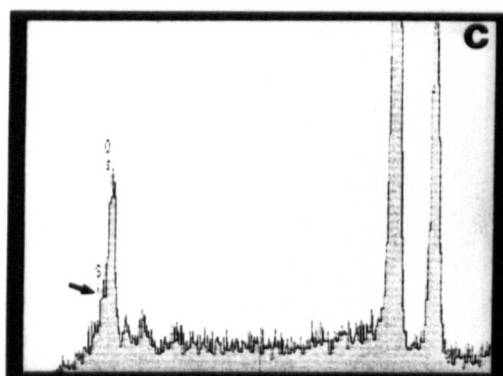
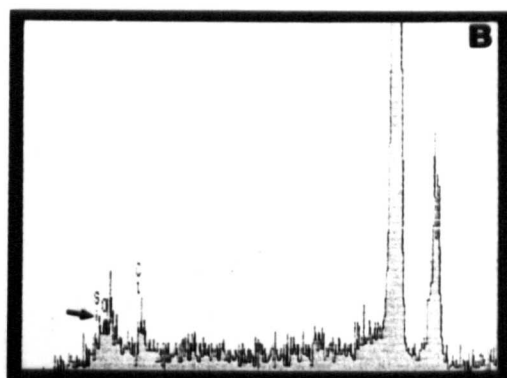
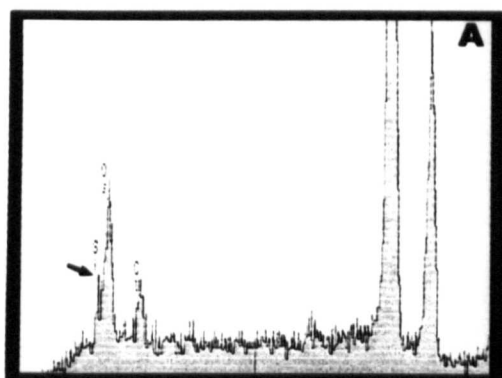
0

keV

10

**Figure 4.7.** X-ray microanalysis spectra from root sections embedded in Spur resin mounted on formvar coated copper grids, viewed using TEM. The root sections were from the basal area (i.e. closest to caryopsis) of plants grown for 5 days in Si<sup>-</sup> nutrient solution and then transferred to Si<sup>+</sup> (0.5 mM Si) nutrient solution. The times correspond to the length of time the plant remained in Si<sup>+</sup> solution before harvest. Control plants remained in Si<sup>-</sup> solution.

- A) Spectrum from the ITW of endodermal cells of roots of plants not exposed to Si<sup>+</sup> solution.
- B) Spectrum from the ITW of endodermal cells of roots of plants exposed to Si<sup>+</sup> solution for 3 h.
- C) Spectrum from the radial wall of endodermal cells of roots of plants exposed to Si<sup>+</sup> solution for 12 h.
- D) Spectrum from the ITW of endodermal cells of roots of plants exposed to Si<sup>+</sup> solution for 24 h.
- E) Spectrum from an electron opaque "spot" within the ITW of an endodermal cell of roots from a plant exposed to Si<sup>+</sup> solution for 24 h.
- F) Spectrum from the ITW to one side of the electron opaque "spot", analyzed in E) of the endodermal cells of roots from a plant exposed to Si<sup>+</sup> solution for 24 h.



0 keV 10

0 keV 10

#### 4.3.5. Chemical Analysis For Silica.

Chemical analysis was used to determine total and soluble (and thus deposited) silica in the terms of  $\mu\text{molesSi/gFWT}$  and  $\mu\text{molesSi/DWT}$  of plant tissue and also the concentration of soluble silica in the xylem.

##### Total and soluble Silica.

The total silica content as  $\mu\text{molesSi/gFWT}$  for roots and shoots is presented in table 4.3. Initially, at day 2, there was no significant difference between the total quantity of silica within the root and shoot of the wheat plants (d.f.=4,  $p=0.05$ ,  $t=-1.05$ ). Total silica content of the plant rose sharply by day 4 (root,  $6.532 \mu\text{molesSi/gFWT}$ ; shoot,  $5.688 \mu\text{molesSi/gFWT}$ , no significant difference). Over the time period of 6 - 10 days the shoots contained significantly more total silica than the roots (d.f.=4,  $p=0.05$ , at 6 days,  $t=-8.07$ ; at 8 days,  $t=-4.65$ ; at 10 days,  $t=-13.42$ ). The silica content of the shoot increased significantly over the 10 days of the experiment (d.f.=4,  $p=0.05$ , between 2-4 days,  $t=-11.36$ ; between 4-6 days,  $t=-8.32$ ; 8-10 days,  $t=-4.544$ ) although there was a significant (d.f.=4,  $p=0.05$ ,  $t=2.960$ ) decrease on day 8. After 10 days the shoot contained  $27.132 \mu\text{moles/gFWT}$ . There was a significant increase in the total silica content of the roots between day 2 and 4 (d.f.=4,  $p=0.05$ ,  $t=-3.85$ ). Over the following period up to 10 days, silica content in the root declined although not significantly. On day 10 of the experiment the root contained  $2.03 \mu\text{molesSi/gFWT}$ .

Soluble silica data is also presented in table 4.3, in  $\mu\text{moles/gFWT}$ . This data includes all the soluble silica in the root including that which was present in the xylem. The amount of soluble silica in the shoot was initially  $0.252 \mu\text{moles/gFWT}$  on day 2 and varied over the 10 day experimental period (see table 4.3), but this variation was not significant (d.f.=4,  $p=0.05$ , between 2-4 days,  $t=-2.33$ ; between 4-6 days,  $t=0.90$ ; between 6-8 days,  $t=-2.52$ ; between 8-10 days,  $t=1.43$ ). The shoot contained  $0.554 \mu\text{moles/gFWT}$  by day 10. The root contained less soluble silica than the shoot at any particular time point, although the difference was only

**Tabel 4.3.** The total, soluble and deposited silica (in  $\mu\text{molesSi/gFWT}$ ) of roots and shoots from plants grown in full nutrient solution containing 0.5 mM silica, for up to 10 days. Also included is the silica concentration in the xylem exudate from the above plants.

TIME (DAYS)	ORGAN	TOTAL Si <sup>1</sup> ( $\mu\text{moles/gFWT}$ )	SOLUBLE Si <sup>1</sup> ( $\mu\text{moles/gFWT}$ )	DEPOSITED Si <sup>2</sup> ( $\mu\text{moles/gFWT}$ )	XYLEM CONC. <sup>1</sup> (mM)
2	Root	1.564 $\pm$ 0.071	0.181 $\pm$ 0.173	1.383	2.07 $\pm$ 0.19
2	Shoot	1.894 $\pm$ 0.54	0.252 $\pm$ 0.209	1.642	
4	Root	6.532 $\pm$ 2.234	0.303 $\pm$ 0.04	6.229	2.87 $\pm$ 0.42
4	Shoot	5.688 $\pm$ 0.207	0.559 $\pm$ 0.092	5.129	
6	Root	4.562 $\pm$ 1.675	0.34 $\pm$ 0.037	4.222	5.22 $\pm$ 1.76
6	Shoot	23.451 $\pm$ 3.693	0.510 $\pm$ 0.019	22.941	
8	Root	3.647 $\pm$ 2.081	0.561 $\pm$ 0.282	3.086	3.63 $\pm$ 0.56
8	Shoot	14.707 $\pm$ 3.554	0.935 $\pm$ 0.292	13.772	
10	Root	2.0299 $\pm$ 0.831	0.295 $\pm$ 0.231	1.804	4.31 $\pm$ 0.39
10	Shoot	27.132 $\pm$ 3.4131	0.554 $\pm$ 0.358	26.578	

<sup>1</sup>n=3

<sup>2</sup> Calculated data



significant on days 4 (d.f.=4,  $p=0.05$ ,  $t=-4.42$ ) and 6 (d.f.=4,  $p=0.05$ ,  $t=-7.08$ ). On day 2 the root contained 0.181  $\mu\text{moles/gFWT}$  soluble silica. There was a general increase in soluble silica content of the root over the time period, but this was not significant (d.f.=4,  $p=0.05$ , between 2-4 days,  $t=-1.19$ ; between 4-6 days,  $t=-1.18$ ; between 6-8 days,  $t=-1.05$ ; between 8-10 days,  $t=1.26$ ).

#### Silica Within the Xylem.

The xylem sap was collected 90 min. after the removal of the shoot. The concentration of soluble silica in the xylem showed an increasing trend over the 10 days (table 4.3). At day 2 the concentration was 2.07 mM, this increased significantly to 2.87 mM by day 4 (d.f.=4,  $p=0.05$ ,  $t=-3.01$ ). There was variation in the xylem concentration recorded although there was no further significant changes in concentration (d.f.=4,  $p=0.05$ , between 4-6 days,  $t=-2.25$ ; between 6-8 days,  $t=1.49$ ; between 8-10 days,  $t=-1.73$ ). The mean average silica concentration in the xylem over the growth period was 3.6 mM.

#### Silica content on a dry weight basis.

Total silica content in the roots, in terms of  $\mu\text{moles/gDWT}$ , is expressed graphically in figure 4.8. Initially, silica content was high in roots, 41.24  $\mu\text{molesSi/gDWT}$  at day 2. The general trend of silica content was a decreasing one. However, there was a large decrease at day 4, to 16.67  $\mu\text{moles/gDWT}$ , which was followed by an increase to 36.73  $\mu\text{moles/gDWT}$  by day 6. There followed a decrease over the next 4 days, and the silica content was 16.82  $\mu\text{mole/gDWT}$  on day 10 of the experiment.

The silica content of the shoot, expressed in terms of  $\mu\text{moles/gDWT}$ , increased over the experimental period of 10 days (fig. 4.9). On day 2, the silica content was 13.24  $\mu\text{moles/gDWT}$  which increased to 41.94  $\mu\text{moles/gDWT}$ . This increase was maintained until day 10 when the silica content was 138.99  $\mu\text{moles/gDWT}$ .

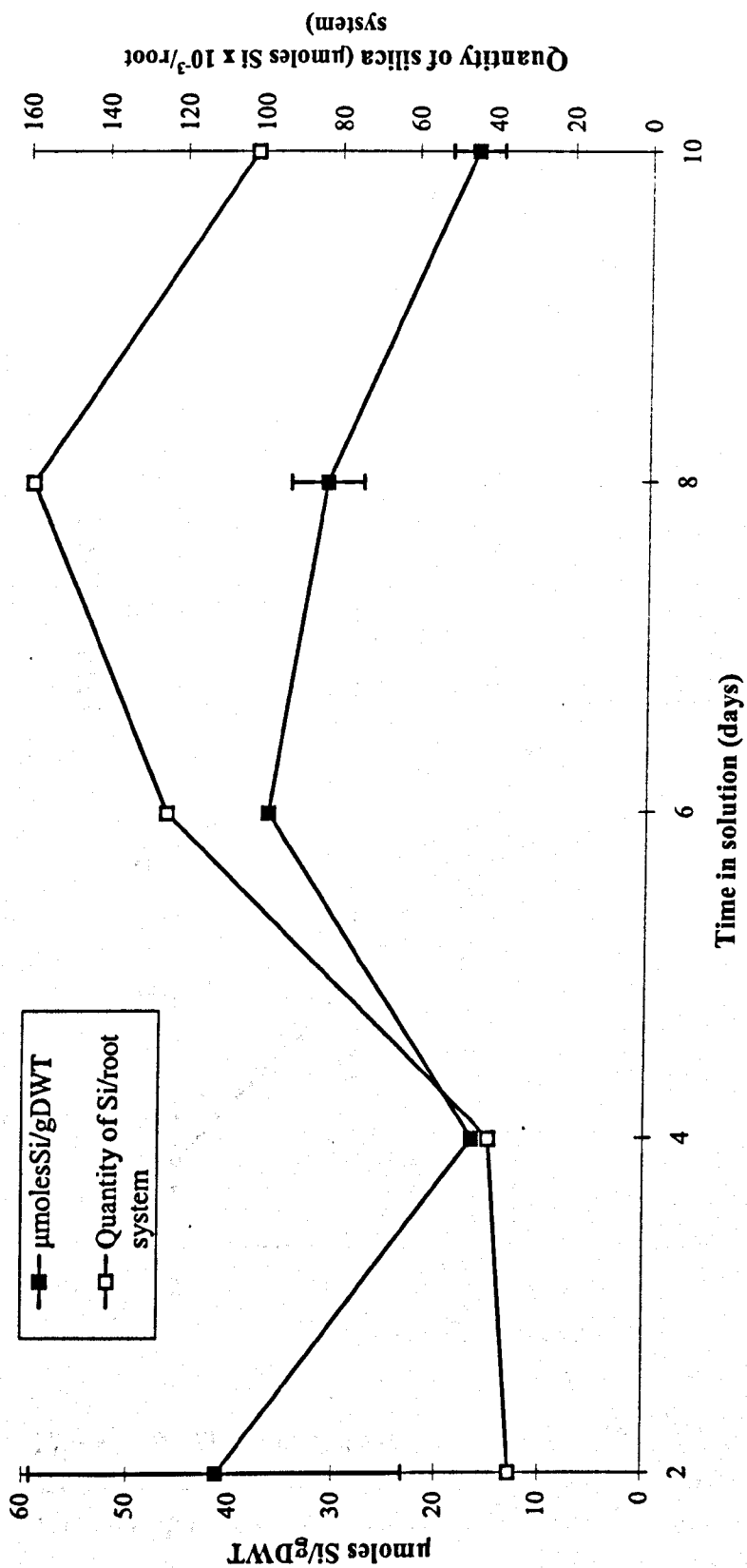
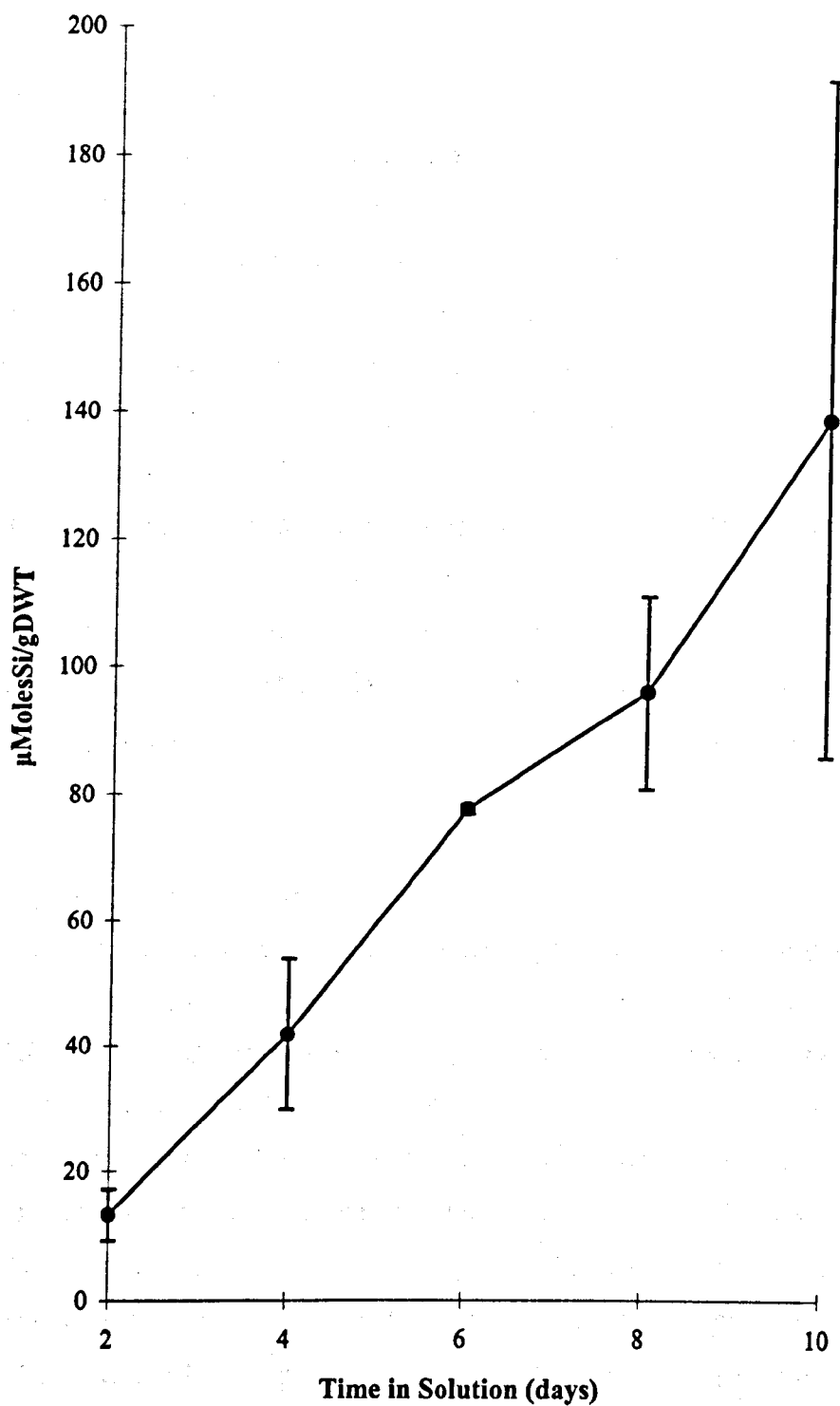


Figure 4.8. The quantity of silica in the roots of wheat plants grown in 0.5 mM silica solution for up to 10 days. The results are expressed as  $\mu\text{moles Si/g DWT}$  ( $n=2, \pm \text{std.}$ ) and  $\mu\text{moles Si} \times 10^{-3}/\text{root system}$ .



**Figure 4.9.** The total silica (in  $\mu\text{molesSi/gDWT}$ ) contained in the shoots of plants grown in full nutrient solution containing 0.5 mM silica for up to 10 days ( $n=2$ , mean average  $\pm$  std.).

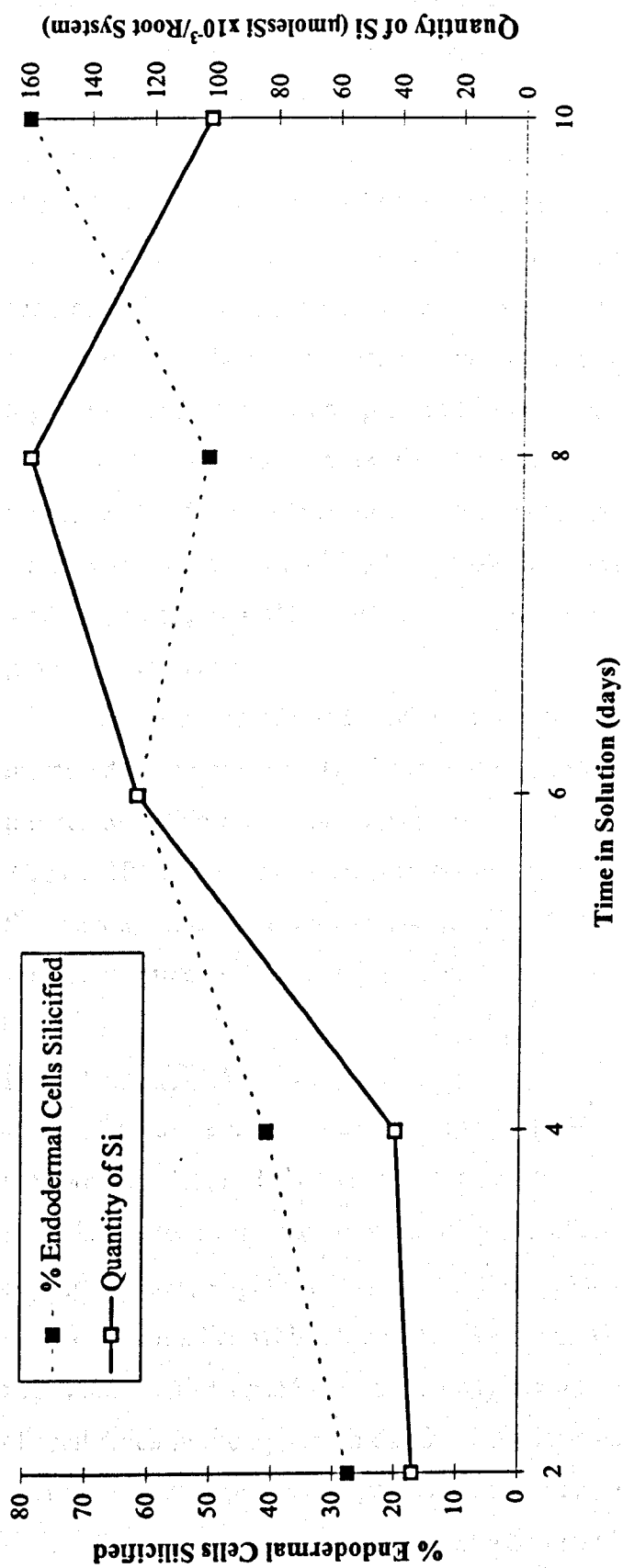
#### 4.3.6. Calculated Results.

##### Silica per Root System.

Using results obtained in chapter 3 it was possible to calculate the quantity of silica present per plant root system using DWT data (fig. 4.8). At day 2, the root system per plant contained 0.034  $\mu\text{moles Si}$ , there was a slight increase to 0.0402  $\mu\text{moles Si}$  by day 4. This value then increased greatly to 0.124  $\mu\text{moles Si}$  by day 6 and again on day 8 to 0.159  $\mu\text{moles Si}$ . There is then a slight decrease by day 10 to 0.102  $\mu\text{moles Si}$ . This data can be compared to silica/gDWT (fig. 4.8). It appears that although there is a general decrease in the silica content expressed as  $\mu\text{moles/gDWT}$ , there is an increase in the amount of silica contained within the root system of the plant. This also compares well with data presented for the percentage of endodermal cells silicified (fig. 4.1). Both silica content per root system and percentage cells silicified increased over the 10 day growth period (fig. 4.10).

##### Deposited, Soluble and Xylem Silica.

Further analysis of the data can be achieved by expressing the data more meaningfully as  $\mu\text{moles Si/root system}$  and the division of the silica into soluble, deposited and xylem silica compartments. To perform these calculations the  $\mu\text{moles/gFWT day}$  (tab. 4.3) and the FWT data of roots from chapter 3 (appendix. I, tab. 2) were used. To determine the  $\mu\text{moles}$  of Si in the xylem vessels required the concentration data in table 4.3, root length data from chapter 3 (appendix. I, tab. 1) and an average xylem vessel diameter obtained by measuring the vessels in root sections prepared for section 4.2.1. Several assumptions had to be made to obtain the xylem data. These included; that the xylem is a hollow uniform cylinder that extends the whole length of the root; that each plant had three roots which had one central metaxylem vessel each; and they ignored any contribution of smaller xylem vessels. These assumptions will be discussed in the discussion section. A sample calculation is presented in appendix III. The



**Figure 4.10.** A comparison of the percentage endodermal cells silicified at the base of the root, as determined using toluidine blue, and the quantity of silica within the root system (in  $\mu\text{moles} \times 10^{-3}$  per root system, calculated from DWT data). The plants were grown in full nutrient solution containing 0.5 mM silica for up to 10 days.

results of these calculations to determine soluble, deposited and xylem silica within the root are presented in figure 4.11.

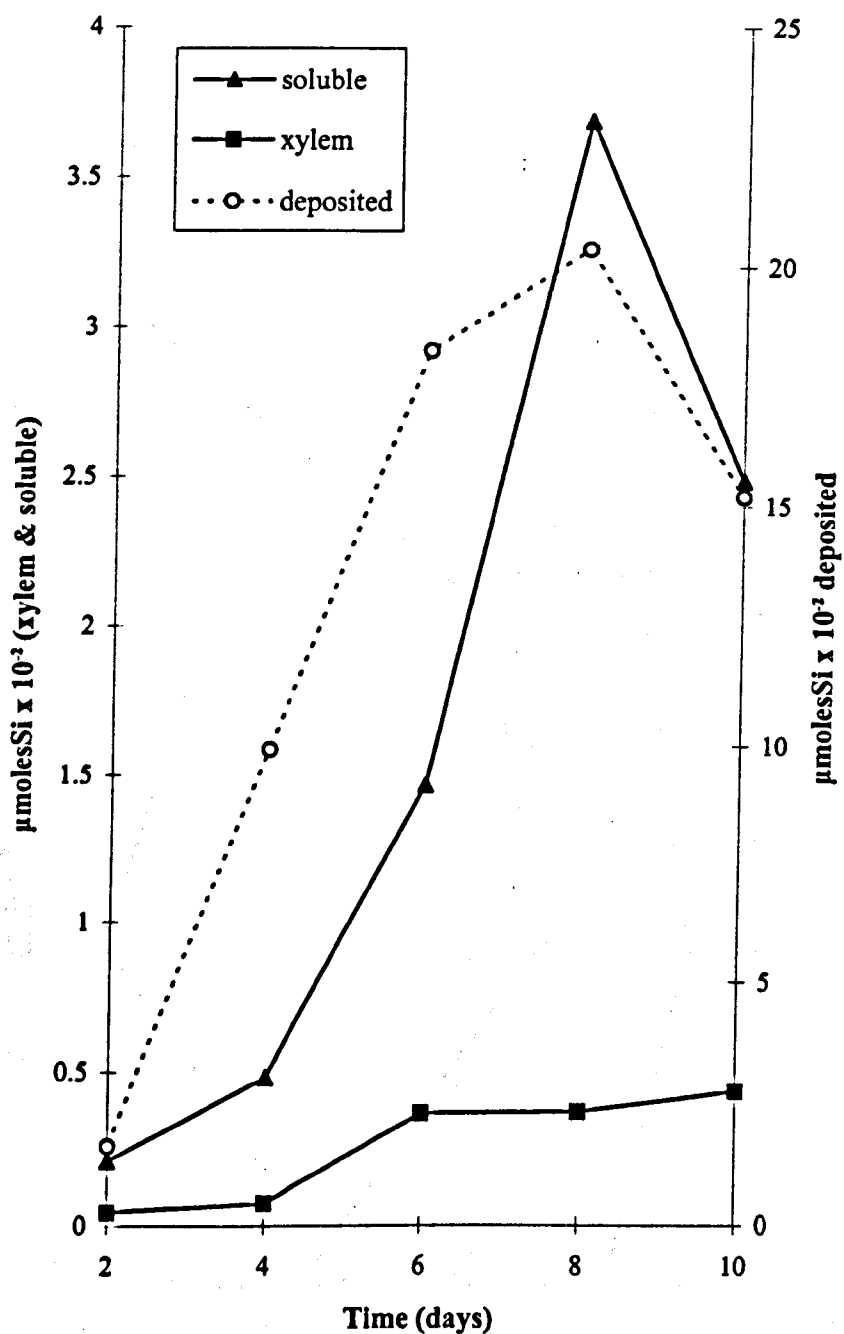
Within the root, the deposited Si showed an increase from  $1.60 \times 10^{-2}$   $\mu\text{moles Si}$  on day 2 to  $20.37 \times 10^{-2}$   $\mu\text{moles Si}$  by day 8. There was a slight decrease on day 10 to  $15.2 \times 10^{-2}$   $\mu\text{moles Si}$ . The soluble silica, of the root, data presented is the silica which was reactive with the molybdate reagent without depolymerization minus the amount of silica calculated to be contained in the xylem. From figure 4.11, it can be seen that there was a general trend of increasing  $\mu\text{moles Si}$  which was soluble. Initially there were  $1.661 \times 10^{-3}$   $\mu\text{moles Si}$  on day 2 which increased to  $4.096 \times 10^{-3}$   $\mu\text{moles Si}$  by day 4. However on day 6, there was a great increase in silica which was soluble, to  $1.104 \times 10^{-2}$   $\mu\text{moles Si}$ . This was followed by a further increase to  $3.316 \times 10^{-2}$   $\mu\text{moles}$  on day 8, decreasing to  $2.048 \times 10^{-2}$   $\mu\text{moles Si}$  on day 10.

The quantity of  $\mu\text{moles}$  of silica within the xylem vessel of the root increased over time. On day 2 the root system contained  $4.389 \times 10^{-4}$   $\mu\text{moles Si}$  within the xylem vessel. There was a slight increase by day 4 to  $7.281 \times 10^{-4}$   $\mu\text{moles Si}$ . However, by day 6, there was  $3.657 \times 10^{-3}$   $\mu\text{moles Si}$ . This appeared to level out, and by day 10 the xylem vessels of the root system contained  $4.369 \times 10^{-3}$   $\mu\text{moles Si}$ .

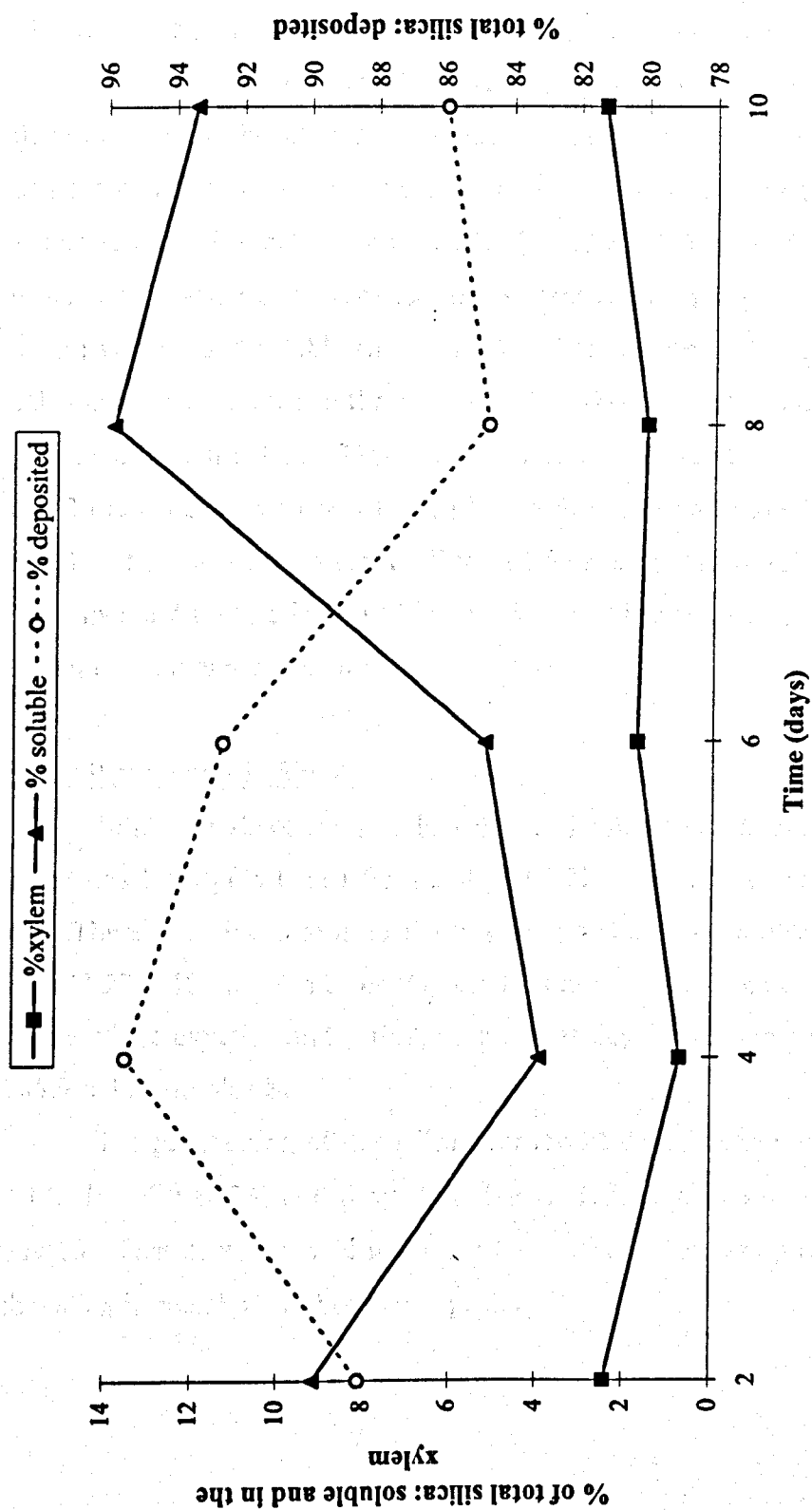
#### Percentage of Total Silica.

The above data can be expressed as percentage of total silica within the root (fig. 4.12). It is clear that the percentage silica present as deposited silica is greater than either soluble or xylem silica. The percentage deposited varied slightly over the time course, but the value was about 90 % of the total silica within the root. The silica which was in the remaining two fractions also varied over the 10 day period. Initially there was 2.42 % of total silica in the xylem on day 2. This decreased to 0.7 % by day 4, after which time there was a gradual increase up to 2.56 % by day 10.

The percentage of total silica which is soluble appears to be very variable over the time period. At day 2 this figure was 9.16 %. This also



**Figure 4.11.** The partitioning of silica within the root between soluble, deposited and xylem fractions, expressed as  $\mu\text{moles} \times 10^{-2}$  per root system (from calculated data)



**Figure 4.12.** The percentage of total silica within the wheat root which is contained within the xylem vessels, present as deposited silica or as soluble silica (excluding silica within the xylem).



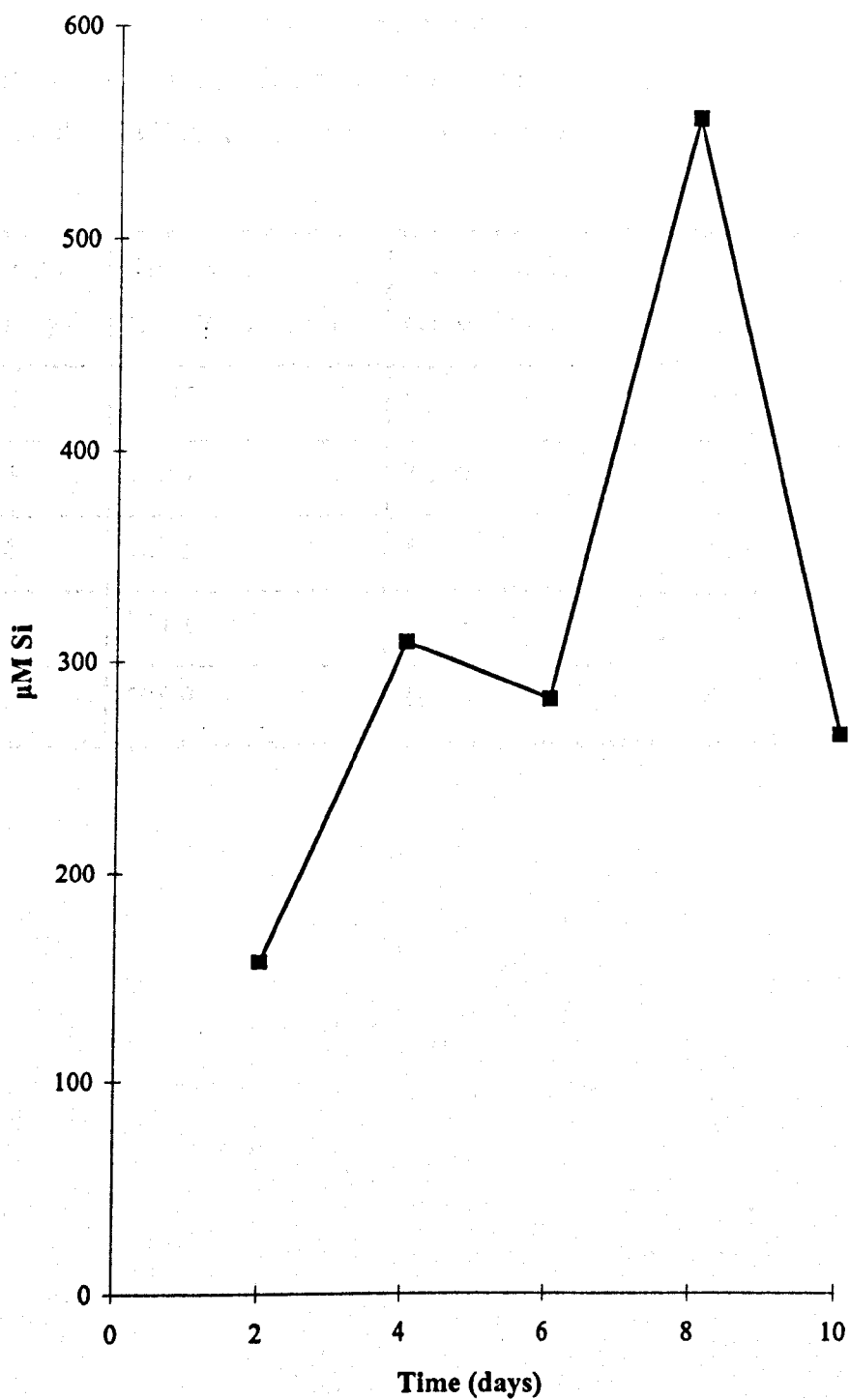
decreased on day 4 to 3.94 %, followed by an increase to 5.24 % by day 6. On day 8 the percentage peaked at 13.84 %, falling to 11.98 % on day 10.

A further calculation which was performed on the data was the determination of the silica concentration in the "root water", i.e. the water contained within the symplast and apoplast, excluding the water in the xylem lumen. The calculation used DWT and FWT data from section 3.3.1 to determine water content of the root system and data from this section on xylem volume and soluble silica ( $\mu\text{molesSi}/\text{root system}$ ). A sample calculation is presented in the appendix III. The calculated data is presented in figure 4.13. This also appeared to be a variable parameter. On day 2 the concentration was  $157.3 \mu\text{M}$  which increased to  $309.13 \mu\text{M}$  by day 4. There was then a rapid fall to  $282.06 \mu\text{M}$  on day 6, followed by a rapid increase up to  $556.56 \mu\text{M}$  by day 8. This decreased to  $265.39 \mu\text{M}$  by the end of the experimental period.

#### Silica Partitioning in Shoots.

Silica per shoot can also be calculated using FWT data from chapter 3 and  $\mu\text{molesSi}/\text{gFWT}$  data from table 4.3. This data is presented in table 4.4. There is an increase in total silica from  $4.482 \times 10^{-2} \mu\text{moles Si}$  at day 2 to  $33.27 \times 10^{-2} \mu\text{moles Si}$  on day 4. This increase continues over the whole of the experimental period, such that at day 10 the shoot contains  $505.9 \times 10^{-2} \mu\text{moles Si}$ .

The partitioning of this silica over the 10 days is presented in figure 4.14. Initially 86.7% is deposited on day 2. This increases up to 98% by day 10. Therefore, it would seem that there was an increasing tendency for the silica in the shoot to become deposited.

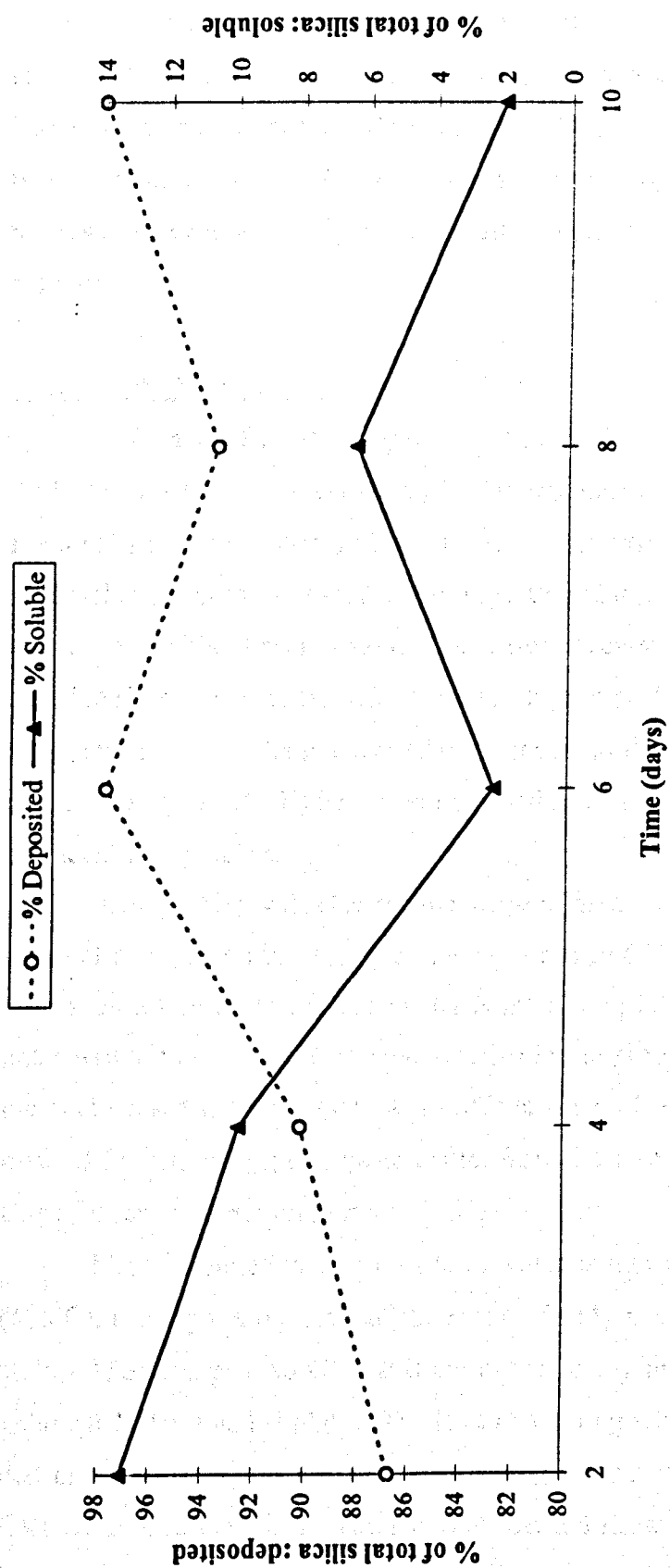


**Figure 4.13.** The concentration of silica in the "root water". This includes the symplast and apoplast but excludes the silica and water contained within the the xylem vessels (from calculated data).

**Table 4.4.**

Silica in the shoots of wheat plants grown for up to 10 days in 0.5 mM silica solution. The data is presented on a plant shoot basis, calculated from the FWT data and  $\mu\text{molesSi/gFWT}$  data

Time (days)	Total silica ( $\mu\text{molesSi} \times 10^{-2}$ )	Soluble silica ( $\mu\text{molesSi} \times 10^{-2}$ )	Deposited silica ( $\mu\text{molesSi} \times 10^{-2}$ )
2	4.482	0.5964	3.886
4	33.27	3.269	30.00
6	167.5	3.643	163.9
8	229.1	14.57	214.5
10	505.9	10.33	495.6



**Figure 4.14.** The partitioning of silica between soluble and deposited fractions within the shoots of wheat plants grown in 0.5 mM silica nutrient solution for up to 10 days.

#### 4.4. Discussion.

Several methods were used to determine the presence of silica in the roots of the wheat plants exposed to silica enriched solution over 10 days. These were staining at the light microscope level, using CVL, MR, and TB, using the fluorescent FITC-APS stain, X-ray microanalysis at the electron microscope level, and a chemical detection method utilizing a molybdate reaction.

##### 4.4.1. Light Microscopy.

CVL and MR did not specifically stain silica in any root sections. Both stains were used successfully by Dayanandan *et al.* (1983) in staining silica cells in the epidermis of rice leaves. They utilized a 50 % sulphuric acid etching step in their methodology, stating that neither CVL or MR would stain without such a step. The reasons they suggested for this were either the surface silica particles were too tightly packed to allow the stain to penetrate, or that the reactive silanol groups on the silica (required for dye binding [Iler, 1979]) had been modified in some way. The etching step corrected this problem.

Acid etching will also remove organic matter from a sample and this removal of organic matter may be the reason why CVL and MR only stain after such a step if the organic matter is interfering in some way. It was not possible to etch the small root samples in the present investigation as the acid caused the sections to wash off the slide. Therefore an alternative method for removing the organic matter from the root sections was used, that of microincineration (Jensen, 1962).

Microincineration burns off the organic matter at high temperatures (400°C), leaving the minerals of the section firmly attached to the glass slide. After this process CVL still did not stain any minerals in the sample, although MR stained slightly. The lack of staining with CVL suggested that organic matter was not blocking staining as its removal did not promote CVL to bind to the silica. The fact that MR did show slight staining, however, suggested that at least some silica was available for staining.

Thus, an alternative, possibly more likely reason, may be that after heating, the silica had been altered such that very specific silica stains, such as CVL, could no longer bind/adsorb to it. Indeed, Shapiro & Kolthoff (1950) investigated the thermal ageing of silica gel. They monitored the ageing of the silica by its decreasing adsorption of MR with increasing temperature. This suggested that heating of silica gel caused changes within the structure which leads to a decrease in staining. Therefore, it would seem that although heating removed the organic matter from the sample, it altered the structure of the silica such that specific silica stains, such as CVL and MR, could not be adsorbed. Jones & Milne (1963) also noted that dry-ashing biogenic opal caused physical changes in the silica, notably "crystallization of a small portion to cristobalite".

Toluidine blue was used by Sangster (1970) to stain silica deposits in the leaves of several grasses. He subjected his samples to wet oxidation, dehydration and clearing prior to staining. This presumably had a similar effect to ashing in removing the organic matter and/or increasing the availability of silanol groups to react with the stain, similar to the etching step used by Dayanandan *et al.* (1983). However, toluidine blue is not specific for hydrated silica (as is CVL and MR) as it also stains quartz, a dehydrated crystalline form of silica. This was observed by Curren (1953). He was using the stain to investigate the fibrogenic activity of quartz dust in mice and guinea-pigs. The quartz dust particles in his sections stained purple, and initially he assumed this was due to a mucopolysaccharide coating on the quartz. Further investigations revealed that the quartz itself was being stained. This result suggested that TB was not specific for hydrated silica, but may be a more general silicon dioxide stain. Therefore, it seemed reasonable that a stain for quartz may stain the altered silica produced during the ashing of plant samples.

TB did indeed stain the minerals in the root sample. The staining was confined to the minerals in the ITW of roots of plants exposed to silica. Staining of fresh sections was to be expected as TB is a metachromatic stain often used to stain acid substances such as nucleic acids and acid

mucosubstances (Sumner, 1988).

#### Development of Silica Deposition.

Silica deposition within the roots in the present investigation increased over the time exposed to the silica solution. It was expected that the percentage of silicified cells would increase over time because silica, once deposited, can not be remobilized within the plant. Therefore, any accumulation of silica is likely to increase with time with the base of the root always displaying greatest percentage of silicified endodermal cells at any one time.

The results presented in this investigation clearly demonstrate the increasing deposition of silica within the ITW over time (fig. 4.1). Also demonstrated is the increasing silica deposition towards the base of the root away from the apex. Similar observations were made by Sangster & Parry (1976a). They studied the development of silica aggregate deposition in the roots of *Sorghum bicolor* exposed to 100 ppm Si (3.6 mM Si) over a 14 day period. Silicon deposition was detected using electron-probe microanalysis and SEM. *S. bicolor* deposits silica in the ITW of the endodermis of its roots but in the form of "discrete accumulations that protrude into the cell lumen", external to the living protoplast. Sangster & Parry (1976a) also demonstrated that there was progressively more silica in the endodermal cells towards the base of the plant. They detected silicification after 1 day of exposure to silica solution. This contrasts with the results from the present investigation which demonstrated, using TB stain (fig. 4.1), that in wheat the endodermis only showed slight silicification after 1 day. It was also observed, by Sangster & Parry, that in *S. bicolor* the silicification of the endodermis became progressively discontinuous towards the apical region of the root. This result is consistent with the present results from wheat which also showed this discontinuity in silicification. The work of Sangster & Parry (1976a) indicated that the basal area of the root had a generally completed silicification of the endodermal cylinder. Again the wheat studied here did not show a

completely silicified endodermis. The "gaps" in the silicification may be due to the presence of passage (or gap) cells in the endodermis. Thin-walled cells were observed in the safranin stained root sections which were thought to be passage cells. *S. bicolor* does not possess passage cells (Sangster & Parry, 1976a) and cereal grasses do not, as a whole, seem to have persistent passage cells (Clarkson, 1991). Thus, the cells observed in the wheat roots would probably not persist during further development. The passage cells, therefore, may be the last cells to be silicified in the roots of wheat plants, and this may be linked to the fact that they mature later than other endodermal cells. There is controversy over the function of passage cells, but if they do indeed do allow movement of solutes into the stele a question is raised as to why does silica not deposit in these cells. A possible reason may be the state of the cell wall. The passage cell wall is relatively unmodified, especially when compared to that of the other endodermal cells, and this seems to strengthen the idea that cell wall modifications are linked to silica deposition. A similar gradient of silicification to that observed in the present investigation, was also noted by Parry & Kelso (1977) in *Saccharum officinarum*.

#### 4.4.2. Fluorescence Microscopy.

TB was a suitable stain over 10 days, but very little silica was detected at 24 hours exposure to silica solution. Therefore, fluorescence techniques were used to try to improve on the sensitivity at the light microscope level. FITC-APS also had the potential of being used for immunogold silica labelling by using anti-FITC antibody labelled with colloidal gold.

However this stain presented problems. It did stain silicified hairs from *P. canariensis* which had the organic matter removed using acid (fig. 4.2). It also stained native hairs, but to a lesser extent. It may be that the organic matter interfered in some way with the staining. Autofluorescence was a major problem with wax embedded samples, but resin sections showed less autofluorescence. However, resin sections of *S. bicolor* with



known silica aggregates (Hodson & Sangster, 1993) did not show specific fluorescent staining. This would support the theory that organic matter was interfering with the staining of the silica and therefore the FITC-APS was rejected as a possible silica stain.

#### 4.4.3. Electron Microscopy.

Conventional TEM studies demonstrated that the ITW of the endodermis was thicker than the adjacent pericycle wall, as expected. Plants exposed to silica enriched nutrient solution appeared to possess electron-opaque areas within the ITW of the endodermis and the xylem vessel walls which were not present in plants which had not been exposed to silica. It was thought that these areas may possibly be sites of silica deposition and were, therefore, investigated.

X-ray microanalysis is one of the most sensitive methods for detecting elements in samples at the electron microscope level. This was applied to resin embedded samples. However, Si was not reliably detected in any samples which had been exposed to silica nutrient solution for up to 24 hours (fig.4.5 - 4.7). Occasional Si peaks were detected in the ITW, as was to be expected when the results from the TB staining was considered, in that silica deposition was slight after 24 hours.

Only the basal regions were analyzed of plants which had been grown in Si- solution for 5 days and then transferred to Si+ for 1 day. It is possible that silica deposition is concurrent with cell wall deposition (see introduction, 4.1). Therefore after 5 days Si- treatment the cell wall in the basal area may already be completed and no silica deposition would occur. Possibly other areas of the root should have been investigated, or alternatively the plant should not have been subjected to the 5 days Si-treatment.

An electron opaque "spot" within the ITW of an endodermal cell was detected which gave a high Si peak (fig. 4.6e). This may suggest that silica deposition within the ITW commences with these small deposits of silica which then act as nucleation sites for further silica polymerization.

This "spot" may have been similar to a primary spherical unit (PSU) which Sangster & Parry (1976c) referred to in their study on *Sorghum bicolor*. The diameter of PSUs was estimated to be about 100 nm, but, photography was not possible in the present investigation, and thus a comparison in size between the "spot" and a PSU was not possible. Bennett (1982) pointed out the diffuse type of wall silicification associated with wheat cultivars contrasts with the aggregates formed in sorghum and this may suggest differences in the formation of silica deposits. The results obtained from this study using X-ray microanalysis suggested that this was not a suitable method for detecting silica in short-term experiments using inhibitors, which was an aim of this investigation.

#### 4.4.4. Chemical Analysis.

The chemical analysis employed in this section of thesis obviously could not give any more information on the sites of silica deposition than was given by the light microscopy study. The quantity of silica in wheat roots was expressed as  $\mu\text{molesSi/gDWT}$  (fig. 4.8) and  $\mu\text{molesSi/gFWT}$  (tab. 4.3). When expressed in these formats, the quantity of silica decreased with time. However, when expressed as  $\mu\text{moles}$  per plant root system (fig. 4.8), there is an increase. This was also deduced from the percentage endodermal cells silicified data (fig. 4.1), which increased over time. From this data it can be concluded that either the root grows faster, or silica is transported to the shoot faster, than silica is acquired from the nutrient solution.

#### Assumptions in the Calculations

A major assumption was that the xylem vessel in each root was a uniform cylinder that extended the whole length of the root. This is clearly not the case. The xylem does not extend to the apex of the root, into the meristem region. Behind this region is an area of differentiation where the xylem begins to form. These regions were included in the root length and the calculations performed assumed that the xylem vessel extended into these regions. It is thought that the length of this area will remain

relatively constant over time. However, it will become proportionally smaller as the root grows longer. Thus, the inaccuracy caused by this assumption will decrease as the root increases in length.

The smaller xylem vessels in the root were completely ignored in the calculation. Only the large central xylem vessel was measured in the root sections. Thus, the calculation assumes that all silica present in the xylem sap was within this vessel and that this accounts for all the water in the xylem. Again, this is clearly not the case. The contribution the smaller xylem vessels make towards the transport of silica is unknown. The inaccuracies from this assumption should remain relatively constant over the time course.

It was assumed that each root contained only one large central xylem vessel. Although this was indeed the case for a the large majority of the root sections observed, the micrographs from the SEM study (fig. 4.4) demonstrate that this cultivar can possess two large xylem vessels. Whether these two smaller xylem vessels contain an equivalent volume to one larger vessel is uncertain and would require further measurements of roots containing two xylem vessels. It is thought, however, that the proportion of roots with two vessels is very small compared to the roots which contain one vessel, as they were rarely observed in the extensive light microscopy study (section 4.2.1). It is believed, therefore, that the assumption of one vessel per root is valid and unlikely to cause large inaccuracies in the calculations.

Lateral roots were not considered in the calculations. The error caused by this assumption would increase as the root ages because the root is more likely to have an extensive system of laterals. The contribution made by any lateral roots is unknown, and thus the inaccuracies caused by this assumption are difficult to determine.

The calculated results from the analysis of silica compartments in the roots clearly show that most of the silica within the root is in the form of deposited silica, with a small amount present as soluble silica (fig. 4.12). Soluble silica refers to silica which is reactive with molybdate reagent,

which are monomers and dimers of silicic acid (Tüma, 1962). This also applies to silica within the xylem vessels. Very little silica, on a total percentage basis is found in the xylem vessels (fig. 4.12). Using X-ray microanalysis Hodson & Sangster (1989c) analyzed frozen samples by SEM, to detect both soluble and deposited silica. Their results showed that Si was localized in the metaxylem and endodermis, but not in the cortex. The Si in the endodermis presumably represents the deposited silica. Their X-ray microanalysis data showed a Si peak when analyzing the pericycle protoplast and a large Si peak associated with the endodermis protoplast. This may be the location of some of the soluble silica which has been detected in the present investigation.

The concentration of silica within the xylem vessels was very high when compared to the levels of silica in the rest of the plant (tab. 4.3). There was an increase from 2.07 mM to 4.31 mM over the over the 10 day growth period. This was similar to the results from Gartner *et al.* (1984). They found that xylem sap collected from 14 day old wheat plants grown in 0.2 mM silica solution had a silica concentration of 2.7-3.2 mM (up to 10 mM in some of the presented data) determined by electron probe analysis, over a 5 hour collection. Also Barber & Shone (1966) found that xylem sap of bean plants had a silica concentration of 4.23-5.06 mM, depending on the silica supplied to the roots. Gartner *et al.* (1984) also determined the concentration of other elements in the xylem exudate. The concentration of silica exceeded that of chlorine (3.0 mM), sulphur (1.9 mM), calcium (1.4 mM) and sodium (0.54 mM). These elements are essential for plant growth (with the exception of sodium), and yet the plant appears to be transporting more silica than these essential elements. Silica concentration was lower than that of potassium (45.5 mM) and phosphorus (8.7 mM) in the xylem exudate.

The wheat plants in this section of thesis were subjected to 0.5 mM silica in the nutrient solution. The concentration of silica in the xylem exudate was up to 4.31 mM. Thus, there was an increase, of over 800%, in concentration of silica between the nutrient solution and xylem sap. This

difference in concentration is usually thought of as evidence of an active step in the transport of the uncharged solute.

The observed concentration of silica in the xylem is thought to be above the theoretical limit for solubility of silicic acid, about 100 ppm (=3.5 mM, Iler, 1955; Birchall, 1978). Birchall (1978) also states that the solubility of silica is reduced in the presence of cations such as calcium, which are present in the xylem. How such high concentrations of silica can be maintained is unclear, although solubility does increase in the presence of dissolved organic matter, mostly due to the capability of silicic acid to form H-bonds. Polyethers, alcohols and amides, for example, may reduce silica polymerization. These and other related compounds are components of the xylem sap of plants (Birchall, 1978).

The silica measured may be complexed in some form, but this complex is either a weak bonding or the silica is free to react with other compounds, as the xylem exudate was analyzed directly for silica with no depolymerization step, and thus the silica in the xylem was reactive with the molybdate reagent. Hartley & Jones (1972) measured silicon in xylem exudate from *Equisetum hyemale* and *Zea mays*. They concluded the silicon in the exudate was in the form of monosilicic acid, based mainly upon results achieved using gel fractionation.

The high concentration in the xylem vessels suggest a high transport rate of silica to the shoots. This was also reported by Jarvis (1987) who observed rapid transport of silica from the roots to the shoots in both ryegrass and wheat. In the present investigation, the shoots show an increasing quantity of silica over time (tab. 4.4) and an increasing percentage of this is deposited. Why this occurs is unclear from the results obtained in the present investigation. It is possible that during the early stages of growth there are very few cells into which the silica can deposit. Blackman (1969) demonstrated that the cell contents disintegrate before silica deposition occurs within potential silica cells of wheat leaves. Therefore it may be that silica is in the soluble form in the leaves until such sites are available. The quantity of soluble silica per gFWT of the shoots

compares well with the results reported by Fox *et al.* (1969). They worked with 15 month old sugarcane (soluble silica was measured using TCA extraction). On average, they detected 18 ppm Si/gFWT (0.64  $\mu$ moles/gFWT). This is a similar value to the quantity detected in the present investigation. Also the free silica content of rye leaves was reported to be 13.4 % of total silica in young leaves and 2.2 % in mature leaves (Blackman, 1966), although this was determined in dried samples. Again this is similar to the results presented in this chapter (86.7 % of total silica was deposited in 2 day old wheat leaves and 98 % in 10 day old leaves). Van Soest (1970), however, reported high levels of soluble silica in various graminaceous plants, up to 22.8 % dry matter in rice hulls, and suggested that this particular fraction accounts for 99 % of the total silica.

#### Root Water Silica Concentration.

This refers to the water present in the root which was not calculated to be present in the xylem vessels, and it includes the water in the symplast and the apoplast of the root. This concentration of silica increased initially, but appears to level off at about 265  $\mu$ M (although there was a peak up to 556  $\mu$ M on day 8, fig. 4.13). There do not appear to be any previous reports concerning the silica concentration in the root tissue water, but the results appear to be lower than concentrations reported in diatoms (see introduction, 4.1).

As this data includes apoplastic and symplastic water, it does not represent the silica concentration in the symplasm. If silica concentration was unaffected by the apoplastic transport through the root, the silica concentration in the apoplast would equal that of the external concentration, ie. 0.5 mM (500  $\mu$ M). This is higher than the calculated concentration for the root water. These results suggest there is a dilution effect of the silica concentration. The concentration within the symplast may be very low which would account for the dilution effect observed. Results reported by Hodson & Sangster (1989c) suggested soluble Si was located in pericycle protoplast and endodermal protoplast, but not in the cortex. This implies

that the distribution of silica in the symplast is not uniform, and therefore the dilution effect may be due to low silica concentrations within the cortical cells.

### Silica Partitioning.

The results presented in the current study can be used to calculate partitioning into various pools (fig. 4.15). The quantity of silica that the plant removes from the solution does not appear to be constant (calculated from total silica per root system and shoot data). Initially the plant obtains  $29.41 \times 10^{-2}$   $\mu$ moles silica from the solution between days 2-4, which increases up to  $142.61 \times 10^{-2}$   $\mu$ moles silica for the period between 4-6 days. This decreased to  $65.1 \times 10^{-2}$   $\mu$ moles silica for the next two day period. Between 8-10 days the plant obtained  $276.7 \times 10^{-2}$   $\mu$ moles silica from the solution. Over the entire experimental period, the plant obtained  $513.82 \times 10^{-2}$   $\mu$ moles silica from the nutrient solution, which originally contained 1000  $\mu$ moles silica (2 litres of 0.5 mM silica). Therefore, the 8 plants in one culture pot removed 41.11  $\mu$ moles silica from the solution. Thus, the change in silica concentration within the pot was 0.02 mM (ie. 958.89  $\mu$ moles silica remaining in 2 litres).

The data presented for quantity of silica per plant for root and shoot in the soluble and deposited form and the total quantity of silica taken up by the plant allowed a calculation to determine silica partitioning. It must be understood that the data represented in figure 4.16 does not represent individual molecules of silica movement, but represents the bulk redistribution of silica within the plant. Figure 4.16 clearly shows that a great percentage of silica is partitioned into the deposited silica pool within the shoot. This trend is consistent over the whole experimental period. In the period between 2 and 4 days, 69.9 % of the total silica taken up by the plant is partitioned into the deposited shoot silica pool. This value increased to 93.25 % during 4 to 6 day period but decreased to 76.8 % on day 6 to 8. During the stage between 8 and 10 days 100 % of the silica taken up by the plant was partitioned into deposited shoot silica. The root

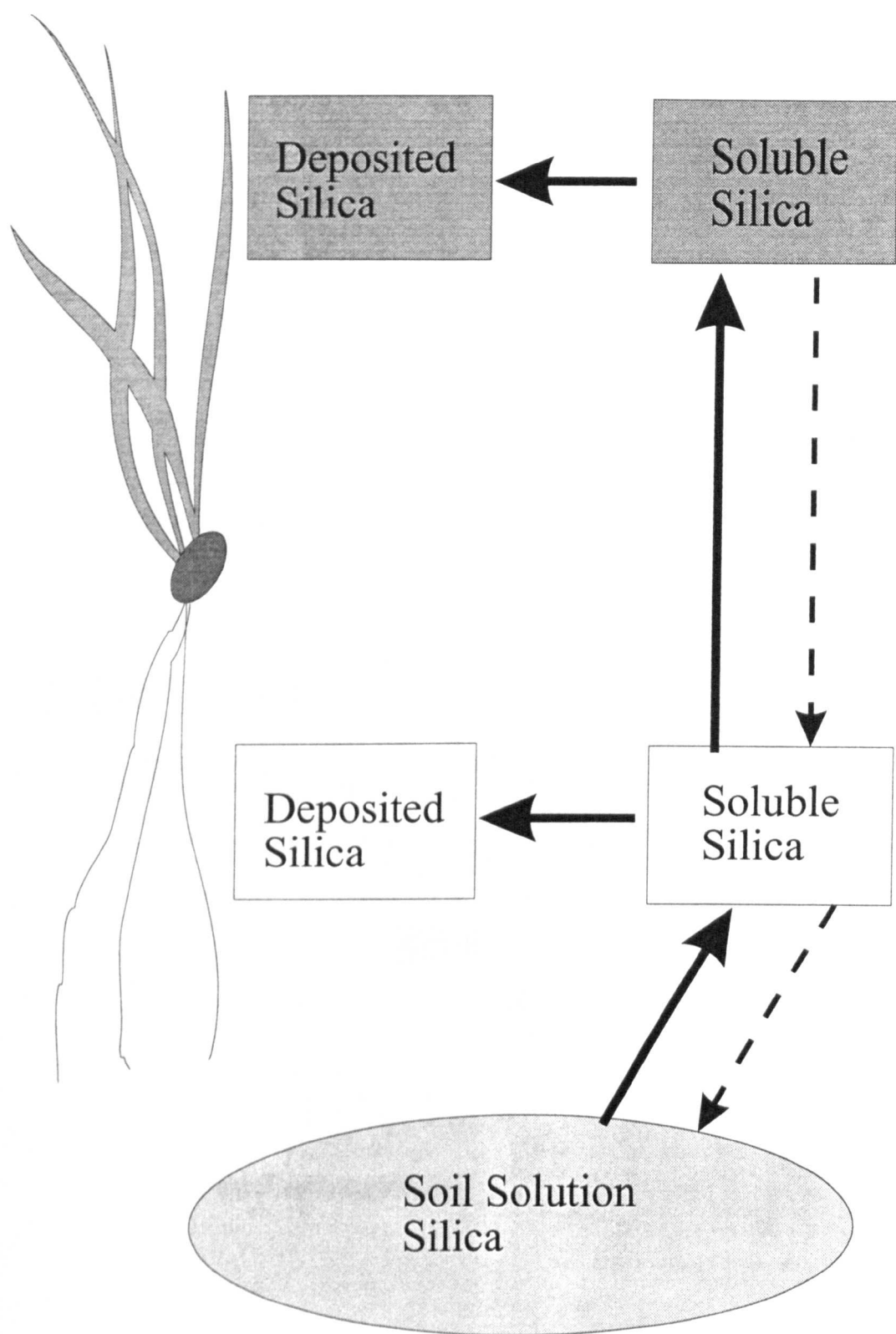
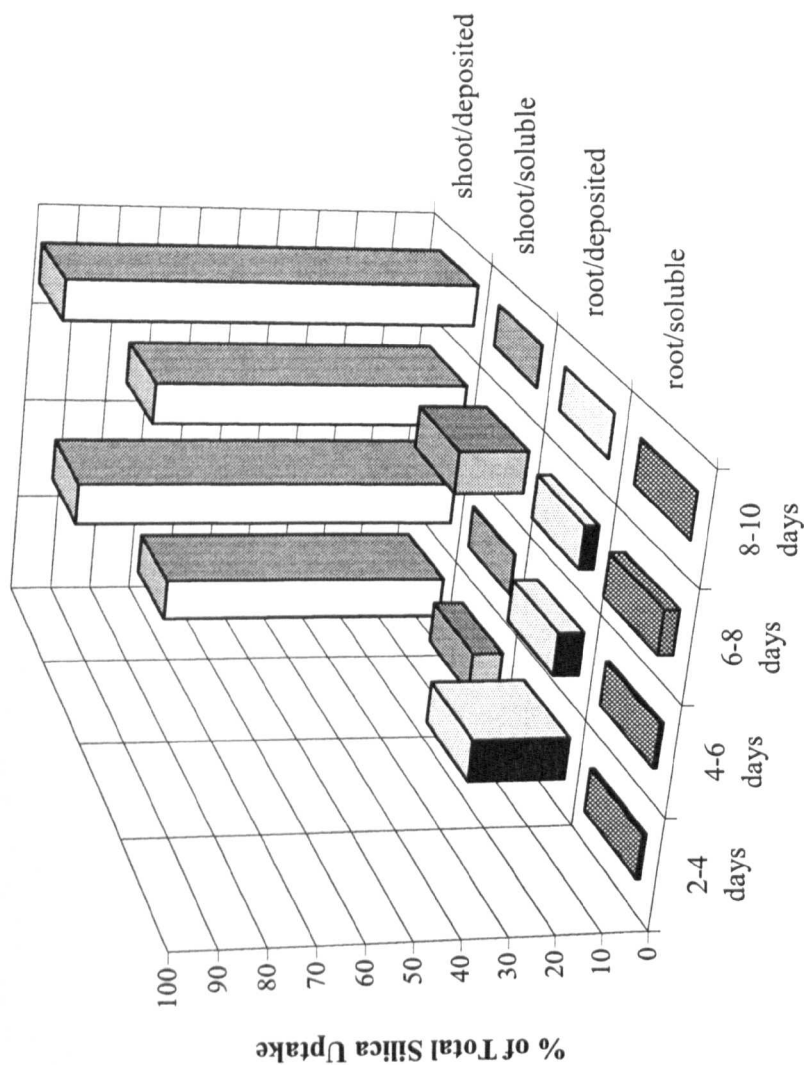


Figure 4.15. The pools of silica within a wheat plant and the possible flow.

—> Likely Silica Flow    - -> Possible Silica Flow





Time in Nutrient Solution

**Figure 4.16.** The calculated partitioning of silica over a 10 day growth period, in 0.5 mM silica nutrient solution.

showed diminished partitioning of silica into the deposited form. This may be due to the available sites for silica deposition within the root may be full. Therefore, additional silica is transported to the shoot where deposition sites may be available. As observed with previous results/calculations the soluble silica concentration appeared to be very variable.

## CHAPTER 5: SILICA UPTAKE AND TRANSPORT.

### **5.1. Introduction.**

Uptake and transport of nutrients is a major area of research in plant physiology. Nutrients within the soil enter the root system and are transferred and loaded into the conducting vessels (phloem and xylem). Once in these vessels, nutrients are distributed throughout the plant and unloaded. This present study concerns the "first half", the uptake into the root and transport to the shoots of an "unusual nutrient", silica. A general review of nutrient uptake and transport will be presented to supply a background against which silica can be compared.

#### General Nutrient Uptake.

Once the root intercepts a nutrient solute there are two pathways within the root through which it can move. These are the apoplastic and symplasmic routes. (Nutrient uptake can occur through leaf surfaces as well as roots, but this section will only be considering root uptake mechanisms.) **Apoplast.** This is the space external to the plasma membrane (PM) of the root cell protoplast. It includes intercellular spaces as well as the cell walls. This "space" has a potential for transporting solutes through the cortical region of the root as water can flow through this space, carrying solutes with it. The cell walls are negatively charged and subsequently attract and adsorb positively charged ions. This adsorption tends to reduce the cation concentration within the apoplastic space, and is important when considering ion uptake. The conditions within the apoplastic space were reviewed by Grignon & Sentenac (1991).

The apoplast pathway is blocked once the cells of the endodermis enter the primary stage (state I) of development. This is characterized by the formation of the Casparian strips, whose structure prevents any further movement within the apoplast (Clarkson & Robards, 1975). Any solute that enters the stele of a differentiated root must enter the endodermal cell itself, i.e. enter the symplast of the plant (see below), in order to traverse the Casparian strip barrier (Flowers & Yeo, 1992). The solutes, once past the

Casparian strips, can then either return to the apoplast or remain within the symplast for its journey across the stele into the xylem vessels which are part of the apoplastic pathway.

Calcium is a nutrient which has been shown to cross the root mainly within the apoplast and when the suberin lamellae are laid down in the state II endodermis transport between cortex and xylem declines rapidly. As calcium is an important messenger within plant cells cytosolic levels are kept low by specific pumps excluding calcium. It has been suggested, however, that calcium may enter the endodermal cells by calcium channels in the outer tangential PM and pumped out, *via*  $\text{Ca}^{2+}$ -ATPase, in the inner tangential PM (see Clarkson, 1993).

**Symplast.** This pathway occurs within the PM of the protoplast. The cells of the root are interconnected by plasmodesmata and can be considered to be a continuous symplastic pathway. Plasmodesmatal structure is complex and still not fully elucidated and will not be considered here, they are thought to be involved in the transport of substances of small molecular mass and electrical currents (Flowers & Yeo, 1992). Once in the symplast, the solute can travel to the xylem, unhindered by the Casparian strip in the endodermis. To enter the symplast of the plant the solute must first cross the plasma membrane of the root cell.

### Membrane Crossing.

The plasma membrane is a lipid bilayer containing proteins (intrinsic and extrinsic) surrounding the cytoplasm of the cell. It functions to maintain the internal environment of the cell (as does the tonoplast of the vacuole) by controlling the import and export of substances. The PM can regulate the transfer of solutes into the cell by various mechanisms outlined below.

**Diffusion.** If the solute is lipid soluble it can dissolve across the PM to enter the cell, providing there is a concentration or electrical gradient present for the particular ion. Very few solutes are lipid soluble and thus

the majority of solutes do not enter the plant in this fashion. Raven (1983) calculated the permeability of  $\text{Si(OH)}_4$  ( $\text{P}_{\text{Si(OH)}_4}$ ) to be low, in the range of  $10^{-10} \text{ ms}^{-1}$ , through a lipid membrane. This is therefore an unlikely route for silica transport.

**Facilitated Diffusion.** Integral PM proteins can form hydrophilic channels to allow diffusion of non-lipid soluble ions into the cell. This again depends on the presence of a concentration and/or electrical gradient. There is evidence that water enters the cell by this method, with proteins (tentatively termed aquaporins) forming water channels through the lipid membrane (see review by Chrispeels & Maurel, 1994).

Both types of diffusion are passive, requiring no metabolic energy expenditure. They both, however, rely upon a concentration or electrical gradient for solute flow. Silica is unlikely to flow down an electrical gradient as it is thought to be taken up as a neutral species (Barber & Shone, 1966). Therefore silica would require a concentration gradient for uptake by passive flow.

**Primary Active Transport.** Often the plant cells accumulate ions against a electrochemical potential and therefore alternative mechanisms are employed that require metabolic energy. These are the active uptake processes involving ion/solute specific ATPase pumps. The processes involved in ion movement across the PM at the molecular level are very complex, and only a simplistic overview is presented in this section. The external moiety of the ATPase protein binds the specific ion and transfers it across the membrane, usually by a change in the configuration of the protein. This ion transfer can be against a concentration gradient, and is usually powered by the breakdown of ATP to ADP. Only two ion specific ATPases located at the PM have been identified in plants (Briskin, Basu & Ho, 1992), the proton ATPase ( $\text{H}^+$ -ATPase) pump and, more recently, a calcium ATPase (reviewed by Briskin, 1990).

**Secondary Active Transport.** This involves the ion/solute crossing the PM *via* symport or antiport with another ion. The cell pumps  $\text{H}^+$  ions out,

across the PM, *via* the H<sup>+</sup>-ATPase pump. This produces an electrochemical gradient across the PM. The outside of the cell is subsequently positive compared to the interior and the [H<sup>+</sup>] is greater on the outside. This is termed the proton gradient. PM H<sup>+</sup>-ATPases have been detected, using immunolabelling, in the epidermal cells, companion cells of the phloem and at lower levels in the pericycle, xylem parenchyma and the endodermis of barley roots (Samuels, Fernando & Glass, 1992). The potential energy "stored" within the proton gradient can be utilized to drive solutes into the cell *via* symporters. The ion/solute required and the H<sup>+</sup> both bind to an integral membrane protein. The "stored" energy in the H<sup>+</sup> gradient is used to power the transfer of the ion in the cell as protons flow down their electrochemical gradient.

Studies concerning nutrients have elucidated their transport pathways. Potassium, although once suspected to enter the symplast *via* a specific K<sup>+</sup>-ATPase pump, is now thought to enter *via* a K<sup>+</sup>-H<sup>+</sup> symport system utilizing the proton gradient established. Schachtman & Schroeder (1994) have isolated from wheat, what appeared to be the high affinity H<sup>+</sup>-K<sup>+</sup> transporter, they termed HKT 1. HKT 1 conferred the ability to transport potassium in mutant yeast cells. It showed great similarity to the properties of the high affinity transporter in plants, such as proton coupled potassium uptake and is localized primarily in the cortical cells of wheat roots. The cDNA of HKT 1 encodes for 10-12 putative membrane spanning domains, but has no consensus sequences for an ATP binding catalytic site, suggesting that HKT 1 does not utilize ATP energy and is a H<sup>+</sup>-K<sup>+</sup> symporter. Recent work by Maathuis & Sanders (1994) supports the theory of H<sup>+</sup>-K<sup>+</sup> symport in plants. It is thought that low affinity transport of potassium is through ion channels.

Phosphate was once thought to enter *via* an OH<sup>-</sup>/P antiporter coupled to the H<sup>+</sup>/K<sup>+</sup> exchanging ATPase (Lin, 1979). However, now anions are considered to enter the cell using symporters coupled to the proton gradient which has been extensively documented in algae systems (see Raven, 1988)

Also, antiporters are present on the cell PM which exchange ions

between the outside and the inside of the cell, again, usually at the expense of energy generated by the proton gradient (for example  $\text{Na}^+/\text{H}^+$ , see DuPont, 1992).

All solutes in the apoplast, which are confronted with the Casparian strip of the endodermis, en route to the stele, must enter the symplast. This must be either by passive diffusion or active transport. Once ions are within the stele they are then transferred to the conducting vessels of the plant, the phloem and the xylem. Other substances (e.g. amino acids etc.) are transported in the xylem and phloem, but only water soluble ions that are transported *via* the xylem will be considered here.

### Xylem Loading.

The nature of ion entry into xylem vessels has been open to question for a long time. One of the main problems concerns the study of xylem parenchyma cells as they are so deeply embedded within the stele of the root tissue. It was first thought that the membranes of the xylem parenchyma cells were inherently "leaky" (Crafts & Broyer, 1938), and that this allowed ions to be released into the xylem vessels. Xylem parenchyma cells (XPC) were observed to accumulate potassium in greater amounts compared to the cortical and other stelar cells in corn roots, supporting the theory that the XPC were responsible for secreting potassium into the xylem vessels. The presence of a carrier mediated transport across the PM was suggested instead of "leaky" membranes (Läuchli, Spurr & Epstein, 1971). In fact, XPC in barley have plasmodesmata and are rich in cytoplasm, rough ER and well developed mitochondria (Läuchli *et al.*, 1974) suggesting that XPC were suitable candidates for solute transport into the xylem. Symplasmic transport was reviewed by Läuchli (1976) who suggested the possibility that ion release to the xylem vessels "is a process different in kind from uptake by the root".

A number of studies have suggested the presence of two electrogenic pumps, one responsible for uptake into the symplast, and the other for transport from the symplast to the xylem vessel. A biphasic response of

the transroot potential to anoxia (in *Plantago* sp.) suggested two spatially separated proton pumps working "back to back", i.e. in opposite directions from the symplast (deBoer, Prins & Zanstra, 1983). It has also been demonstrated that the translocation of potassium into shoots appeared to be regulated independently from potassium uptake into roots (Engels & Marschner, 1992). This provides further support for the presence of two pumps, one involved in transport into the symplast from the external solution, and another involved with transport from the symplast to the xylem.

Additional evidence for an electrogenic pump at the symplast/xylem interface was provided by Clarkson, Williams & Hanson (1984) using a method of perfusing the xylem vessels of excised onion roots. It was found that the XPC could alter the pH of the solution passing through the xylem, demonstrating the presence of a proton absorbing/releasing mechanism. Further to these investigations, it was "evident that the activity of the proton secreting and proton neutralizing mechanisms in the xylem parenchyma controls the movement of other ions across the xylem/symplast boundary" (Clarkson & Hanson, 1986). Clarkson reviewed the situation in 1993, commenting on the suggested inner pump that "there is, however, persuasive evidence that they (stelar parenchyma cells) do possess an H<sup>+</sup>-ATPase". This is supported by the data presented by Samuels *et al.* (1992).

A methodology for isolating protoplasts from the XPC of barley roots using differential enzymatic digestion has been presented by Wegner & Raschke (1994). The protoplasts isolated were then studied using patch clamp techniques. Results suggested salt loading occurred by the simultaneous flow of cations and anions through channels following electrochemical gradients established by the ion uptake processes. They additionally stated that passive salt release required negative electrochemical gradients.

A mutant of *Arabidopsis thaliana*, deficient only in xylem phosphate loading, has been isolated (Poirier *et al.*, 1991). The results suggested that the protein activity required for phosphate loading was absent due to a



mutation at a locus designated *pho* 1. This pump appeared to be specific for phosphate as xylem loading of other nutrients was virtually unaffected suggesting the presence of phosphate transporters at the symplast/xylem interface.

Reports, therefore, propose that solute transport into the xylem vessels is accomplished by the presence of  $H^+$ -ATPases producing an electrochemical gradient, a similar process to that occurring at the external medium/symplast interface.

### Silica Transport.

The above is a general overview of nutrient uptake and transport to the xylem in plants. In the natural habitat of plants, silica is acquired from the soil solution. As silica is found in the aerial organs of plants, as well as the roots, it must be transported through the root to the conducting vessels similar to other solutes.

It is thought that silica is present in the soil solution as monosilicic acid  $Si(OH)_4$  at concentrations up to 1.2 mM (Jones & Handreck, 1965, 1967). At physiological pH monosilicic acid is a predominantly uncharged species and it is in this form, it is assumed, silica is taken up into the plant system. This is an interesting point as most other inorganic plant nutrients are taken up as charged ions, thus making the study of silica almost unique in the field of mineral uptake. Another mineral nutrient which is thought to be taken up as a neutral species is boron in the form boric acid. The review by Raven (1980) suggested boric acid is sufficiently lipid soluble ( $P_{B(OH)_3}$  is in the range  $10^{-5}$  to  $10^{-6} \text{ cm s}^{-1}$ ) to account for the transmembrane fluxes observed. Also the formation of *cis*-diol complexes complicates boron analysis and suggests the possible uptake of borate anions.

Silica is not known to be a nutrient element in plants (except rice, horsetail and diatoms), and few studies have been directed towards elucidating its uptake mechanisms. Many ion uptake studies have relied upon sensitive detection methods for the particular element of interest. Radioisotope tracers are very popular due to their sensitivity, and have been

used extensively. Examples are  $^{86}\text{Rb}^+$  (potassium studies),  $^{32}\text{P}$ ,  $^{45}\text{Ca}$  and  $^{36}\text{Cl}$ . Unfortunately silica does not have a readily available radioisotope,  $^{31}\text{Si}$  has a half-life of 157 min. This may limit studies concerning uptake and transport of silica.

### Diatoms.

These single celled algae have an absolute requirement for silica and their mode of silica uptake has been greatly studied, compared to higher plant studies. Diatoms can maintain a massive concentration gradient of silica against low silica concentrations in the external solution, possibly a 250-fold concentration gradient (Sullivan, 1976). Silica uptake in the marine diatom *Nitzschia alba* was studied, and it was concluded that this diatom species possessed a membrane bound mono-silicic acid stimulated ATPase which may be involved in silica uptake (Hemmingsen, 1971). Further studies on *N. alba* cells and isolated vesicles revealed that Si uptake was inhibited by monensin and gramicidin, both of which decrease the sodium gradient across the membrane. Valinomycin, which decreases the potassium gradient, and nigericin, which promotes  $\text{H}^+\text{-K}^+$  exchange, did not inhibit Si uptake suggesting that Si uptake in this species of diatom required a sodium gradient, possibly by the utilization of a sodium-silica symporter (Bhattacharyya & Volcani, 1980). Research on silica uptake, using  $^{68}\text{Ge}$  (as a tracer for Si), in the photosynthetic freshwater diatom *Navicula pelliculosa* demonstrated that uptake was related to cell number, pH, temperature, light and the cations within the medium, especially sodium. Uncouplers and inhibitors of oxidative phosphorylation reduced uptake as did sulfhydryl blocking agents. Valinomycin, a potassium ionophore, also inhibited uptake, implying a requirement for a potassium gradient (Sullivan, 1976). This is in conflict with the hypothesis proposed by Bhattacharyya & Volcani (see above). However, this apparent contradiction might be due to the fact that *Nitzschia alba* is a marine diatom, whereas *Navicula pelliculosa* is a freshwater diatom.

## **Silica Transport in Higher Plants.**

A review of the literature concerning Si uptake in higher plants reveals a varied situation with some plants actively excluding Si while others actively acquire it (tab. 5.1). The excluding plants tend to be dicotyledonous. Whether there are specific pumps or an impermeable barrier to exclude Si is unknown (Jones & Handreck, 1969). Many of the plants are in the category of passive silica uptake. To determine if uptake is passive or active a common method is to calculate the Transpiration Stream Concentration Factor (TSCF) (Russell & Shorrocks, 1959) for silica or similar ratio. This is defined as:

$$TSCF = \frac{\text{Concentration of Si within the Transpiration stream}}{\text{Concentration of Si within the External Solution}}$$

If this value equals unity it indicates that the quantity of Si in the shoot can be solely accounted for by mass flow of Si within the transpiration stream. If TSCF is calculated to be less than unity, Si is excluded from the plant. When TSCF exceeds unity it is concluded that processes other than mass flow must be occurring in the plant.

**Transpiration Effects.** Barber & Shone (1966) grew barley in nutrient solutions containing 1.1 mM Si to explore the effect of transpiration on the uptake of silica. They observed that although plants in low humidity conditions transpired 2.5 - 3 times more than plants in a moist environment, there was no significant difference in the quantity of silica entering the shoots. This implied that silica did not enter passively within the transpiration stream. However, results from investigations using oats (Jones & Handreck, 1965) suggested that this species acquired silica passively, and that the quantity of silica within the plant was proportional to the concentration of silica supplied by the soil. This led to the idea of using Si content in cereal crops as an indication of water usage of the plant,

**Table 5.1. A Summary of Silica Investigations**

Species	Culture	Si Conc.	Uptake Mode	Reference
<i>Triticum aestivum</i>	Water culture	0.21 mM	Active	Rothbur & Scott, 1957
<i>Hordeum vulgare</i>	Excised roots	0.66 - 8.83 mM	Initial entry - passive; Transfer across - active	Barber & Shone, 1964
<i>Avena sativa</i>	Pot Expt.	1.1 - 0.18 mM	Passive	Jones & Handreck, 1965
<i>Hordeum vulgare</i>	Water culture	1.1 mM	High humidity - active; Low humidity - passive	Barber & Shone, 1966
<i>Phaseolus vulgaris</i>	Water culture	0.07 - 3.5 mM ----- 6 mM	Active ----- Excluded	Barber & Shone, 1966
<i>Trifolium incarnatum</i>	Water culture	0.033 - 1 mM	Excluded	Jones & Handreck, 1969
Soybean	Water culture	0.01 mM, 0.5 mM & 2.7 mM	Passive at low Conc, excluded at high Conc.	Van der Vorm, 1980
Sunflower	Water culture	0.01 mM, 0.5 mM & 2.7 mM	Active at low Conc., excluded at high Conc.	Van der Vorm, 1980

continued overleaf.

Table 5.1 continued

Species	Culture	Si Conc.	Uptake Mode	Reference
<i>Triticum aestivum</i>	Water culture	0.01 mM, 0.5 mM & 2.7 mM	Active at low Conc., excluded at high Conc.	Van der Vorm, 1980
Sugar cane	Water culture	0.01 mM, 0.5 mM & 2.7 mM	Active	Van der Vorm, 1980
<i>Oryza sativa</i>	Water culture	0.01 mM, 0.5 mM & 2.7 mM	Active	Van der Vorm, 1980
<i>Lolium perenne</i>	Water culture	0.34 mM & 0.71 mM	Active	Jarvis, 1987
<i>Triticum aestivum</i>	Water culture	0.71 mM & 1.42 mM	Active	Jarvis, 1987
<i>Hordeum vulgare</i>	Greenhouse pot	unknown	Active	Walker & Lance, 1991
<i>Hordeum vulgare</i>	Field Pot	unknown	Passive	Walker & Lance, 1991
<i>Triticum aestivum</i>	Field	average ca. 1.4 mM	Active	Mayland, Wright & Sojka, 1991
<i>Agropyron desertum</i>	Field Pot	unknown	Active	Mayland <i>et al.</i> , 1993

assuming that the soil silica concentration was known and was stable. Reports have suggested, however, that this is unsuitable for wheat (Schultz & French, 1976; Jarvis, 1987; Mayland, Wright & Sojka, 1991), *Lolium perenne* (Jarvis, 1987) and *Agropyron desertorum* (Mayland *et al.*, 1993). Considering these reports, the relationship of silica uptake to water use should be treated with caution as it is only applicable to plants which are known to have passive silica uptake. Further complicating the situation, results suggested that barley in greenhouse conditions actively acquires silica, but when in the field and "under higher evaporative demand", silica accumulates passively (Walker & Lance, 1991). Few plants have been shown to actively acquire silica regardless of environmental conditions. Rice is one of these, it has clearly been shown to actively take up silica. Most studies have been performed by Japanese workers and these were reviewed by Okuda & Takahashi (1965).

**Concentration and Temperature Effects.** The mode of silica uptake is usually dependent on the concentration of silica supplied. Van der Vorm (1980) performed an extensive investigation on the effect of silica concentrations on silica uptake in various plant species (see tab. 5.1). The plants were grown in 0.01, 0.5 and 2.7 mM Si in nutrient solutions at pH 5.2. It was concluded that Si uptake displayed features of active, metabolic uptake, i.e. preferential absorption at low concentration and exclusion at high concentration. It was suggested that the processes by which Si was taken up do not differ basically from those known for other elements.

The effect of temperature on silica uptake on barley was investigated by Barber & Shone (1966). The roots were kept at 25 or 5 °C for 24 and 48 h.. There was no significant difference in transpiration, but the quantity of silica transported to the shoot decreased when subjected to 5 °C, suggesting that silica was taken up actively.

**Inhibitor Effects.** The use of metabolic inhibitors is common in the elucidation of transport pathways, and a few investigations have applied

such methods to silica. However, the inhibitors used in the past were very general metabolic inhibitors, and thus specific data on uptake processes was not obtained. The use of inhibitors has a number of difficulties. The time of exposure is difficult to determine, and dependent on the material being studied. Isolated membranes may only require minutes for the inhibitor to affect its target, whereas the time is greatly extended when considering the whole root system. Also the plant may be able to inactivate the inhibitor over time or sequester it within cellular compartments. Various metabolic inhibitors have been used in studying Si uptake in rice (reviewed by Okuda & Takahashi, 1965). Sodium fluoride (NaF), 2,4-dinitrophenol, d-glucosamine, monoiodo-acetate, 2,4 dichlorophenoxyacetic acid and sodium cyanide (NaCN) all inhibited silica uptake. It was concluded that energy from respiration was required for Si uptake by rice, as metabolic inhibitors inhibited silica transport (Mitsui & Takatoh, 1963b). Similar results were reported by Barber & Shone (1966) working on barley. As stated above, these reports used general metabolic inhibitors to investigate silica uptake which did not provide data on specific transport processes, therefore the present investigation will build upon these results by using more specific inhibitors to study silica uptake in wheat seedlings. The specific inhibitors to be used are sodium orthovanadate, a commonly used ATPase inhibitor, erythrosin B, an ATPase inhibitor with potential use *in vivo* (Cocucci, 1986), diethylstilbestrol, another ATPase inhibitor which has been used extensively on oat roots (Balke & Hodges, 1979a, b, and c), nigericin, an ionophore which promotes the exchange of protons and potassium across membranes and has been used for studying silica uptake in diatoms (Bhattacharyya & Volcani, 1980) and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), an proton ionophore.

The simplest of questions about Si transport in higher plants remain unanswered, such as whether there is a pH optimum for Si uptake (most studies use pH of about 5.0) as with other uptake systems. What is the concentration optimum for Si uptake ? This investigation is intended as a preliminary study on the process of silica uptake by young wheat seedlings.

Areas of investigation are the effects of the pH of the nutrient solution and Si concentration supplied to the plants, in the range 0-5 mM. Passive versus active uptake will also be calculated, and the effect of specific inhibitors, of ATPases and the proton gradient, on silica uptake and transport determined (including an initial study using excised roots). Potassium and phosphate content were also analyzed after exposing the plants to specific inhibitors to determine the effect of the inhibitors on solutes known to be actively taken up. The five inhibitors to be considered in this investigation are sodium orthovanadate, erythrosin B (EB), diethylstilbestrol (DES), nigericin and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP). The first three are ATPase inhibitors, and the latter two are ionophores.



## **5.2. Materials & Methods.**

### **5.2.1. Experimental Design.**

All plants for this section (except part 5.2.4) were grown individually in 10 ml plastic test-tubes (culture tubes), supported by foam bungs, in the same nutrient solution and conditions as before (see section 3.2.1). The tubes were held upright in polystyrene trays and covered with aluminium foil to prevent light reaching the roots. The tubes were placed randomly in the trays. All the tubes were washed in "Micro" (International Products Corporation), acid washed (0.1 M HCl), and rinsed three times in distilled water after each use if they were to be used again.

Experiments using the above methodology investigated the effects of external silica concentration, pH of the nutrient solution, and specific inhibitors, on the silica content of wheat plants over 24 hours.

### **5.2.2. The Effect of Silica Concentration.**

The concentration of added silica was varied between 0-5 mM, and the pH of the solutions was adjusted to  $5.2 \pm 0.1$  using HCl and NaOH. The plants were transferred to the experimental solutions after a 4 day germination period. They were harvested 24 h. after the transfer to the culture tubes. The roots and shoots were dried and analyzed for total silica as in section 4.2.7.

### **5.2.3. 24 Hour Time Course of Silica Uptake.**

Plants were transferred to Si<sup>-</sup> solution and Si<sup>+</sup> solution (2 mM added silica) after the 4 day germination period. The pH of the solutions was adjusted to pH  $5.2 \pm 0.1$  using HCl and NaOH. The plants were harvested 3, 6, 9, 12 and 24 h. after being transferred to the nutrient solution. The roots and shoots were dried and analyzed for total silica as in section 4.2.7.

### **5.2.4. The Effect of pH on Silica Uptake.**

Solutions were prepared without added silica (Si<sup>-</sup>) and with 2 mM added silica (Si<sup>+</sup>) and were set at pH 3.2 - 9.2 (at 1 pH unit intervals) using

HCl and NaOH. The plants were grown in these solutions in 2 l water-culture pots (as in section 3.2.1) to reduce the effect of the plants themselves altering the pH of the solution while they were growing in it. The plants were grown for 24 h. and then the roots and shoots were harvested, dried and analyzed for total silica as in section 4.2.7. The pH of the nutrient solution was measured after the 24 h. growth period and had not changed from the initial set value.

#### 5.2.5. Transpiration Experiment.

100 culture tubes were set up with full nutrient solution (pH was  $5.2 \pm 0.1$  using HCl and NaOH). Fifty of these tubes had silica added to give a concentration of 2 mM (Si+). Plants were grown in half of these tubes (i.e. 25). The other half did not have plants, with only the foam bung support present, to act as a control to determine the amount of evaporation that occurred. The other 50 tubes had no silica added (Si-). Again half these tubes contained plants, and the other half had only foam bungs.

All of the tubes containing nutrient solution were weighed, as was each individual plant. The plants were then placed in the tubes and grown in the growth cabinet (see section 3.2.1) for 24 h. After this time all the tubes and plants were reweighed to determine the amount of water loss from the tubes by evaporation and the amount of water used by the plants.

The roots, shoots and grains of the plants were then dried in a 60°C oven for 48 h. The total silica content of these samples was determined as described in section 4.2.7 and the amount of water transpired could be calculated from the weight difference. The theoretical quantity of silica taken up can be calculated from the volume of water transpired and the silica concentration in the nutrient solution and compared to the actual amount of silica within the plant. The results should indicate whether silica enters the plants passively in the transpiration stream or is actively taken up from the nutrient solution (see introduction, 5.1., for calculating TSCF).

### 5.2.6. The Effect of Inhibitors on Ion Content.

Five inhibitors were used to investigate silica uptake and transport in the wheat plant. These were 3 ATPase inhibitors; sodium orthovanadate (obtained from BDH), erythrosin B (EB), diethylstilbestrol (DES), a potassium-proton ionophore, nigericin and a proton ionophore, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), (all obtained from Sigma Chemicals), in the concentration ranges 0-100  $\mu\text{M}$ , 0-1  $\mu\text{M}$ , 0-100  $\mu\text{M}$ , 0-10  $\mu\text{M}$  and 0-100  $\mu\text{M}$  respectively. The inhibitor was added to the Si- (no added silica) and Si+ (2 mM silica added) nutrient solution (pH was set to  $5.2 \pm 0.1$  using HCl and NaOH). When the inhibitor had to be initially dissolved in an alcoholic solution, ethanol was added to all the nutrient solutions to give an equal concentration. The plants were grown for 24 hours in plastic test-tubes (see 5.2.1) and then harvested. The roots and shoots were dried and the total silica content was analyzed as in section 4.2.7 (standard curve presented in appendix IV). Phosphate and potassium contents were also analyzed in both roots and shoots.

#### 5.2.6a. Phosphate Analysis.

The spectrophotometric analysis was after Allen, Grimshaw & Rowland (1986). The dried sample was prepared as for total silica analysis (section 4.2.7.). This was then diluted 25 times. 200  $\mu\text{l}$  of diluted sample was then added to a plastic test tube (capacity 4 ml) and diluted with 1000  $\mu\text{l}$  of ultra pure water. To this, 80  $\mu\text{l}$  of molybdate reagent (2.5 g  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 0.05 % sulphuric acid) was added, followed by 80  $\mu\text{l}$  of stannous chloride solution (0.02 g  $\text{SnCl}_2 \cdot 4\text{H}_2\text{O}$  in 10 ml 2 % HCl). Ultra pure water (640  $\mu\text{l}$ ) was finally added (the tube was whirl-mixed after each addition) and the colour allowed to develop for 30 mins. Absorbance was measured at 700 nm using a Perkin Elmer Lambda 3B spectrophotometer. All of the standards (prepared from  $\text{KH}_2\text{PO}_4$ ) had an equivalent quantity of blank acid-extraction added to account for the acid in the prepared samples (standard curve presented in appendix IV).

#### 5.2.6b. Potassium analysis.

The prepared samples (as in section 4.2.7, for total silica determination) were diluted 1:200 in plastic volumetric flasks and analyzed for potassium using a Corning Flame photometer 410. Standards were prepared from  $\text{KH}_2\text{PO}_4$ . All the standards had an equivalent quantity of blank acid-extraction added to account for the acid in the prepared samples (standard curve presented in appendix IV).

#### 5.2.6c. Excised Roots.

Preliminary experiments were also commenced using excised roots (method based on Epstein, Schmid & Rains, 1963). The roots were removed from plants after 4 days germination period, blotted briefly on Whatman 40 ashless filter paper and then about 100 mg of fresh root material was accurately weighed and placed in "cheese cloth" which was closed with a bag closure tie. The roots were then incubated at 20°C for 3 h. on a rocking table in one experimental solution. The experimental solutions were as follows:

Experimental solution 1. Full nutrient solution with no added silica (Si-) and no inhibitor.

Experimental solution 2. Full nutrient solution with 2 mM added silica (Si+) and no inhibitor.

Experimental solution 3. Full nutrient solution, Si- with 1  $\mu\text{M}$  EB.

Experimental solution 4. Full nutrient solution, Si+ with 1  $\mu\text{M}$  EB.

After three hours the bags were washed by blotting on ashless filter paper and placing in cold nutrient solution, on ice, without silica for 30 mins. After this time the roots were analyzed for total silica content as fresh samples (see section 4.2.7, soluble silica determination).

### 5.3. Results.

#### 5.3.1. The Effect of Silica Concentration.

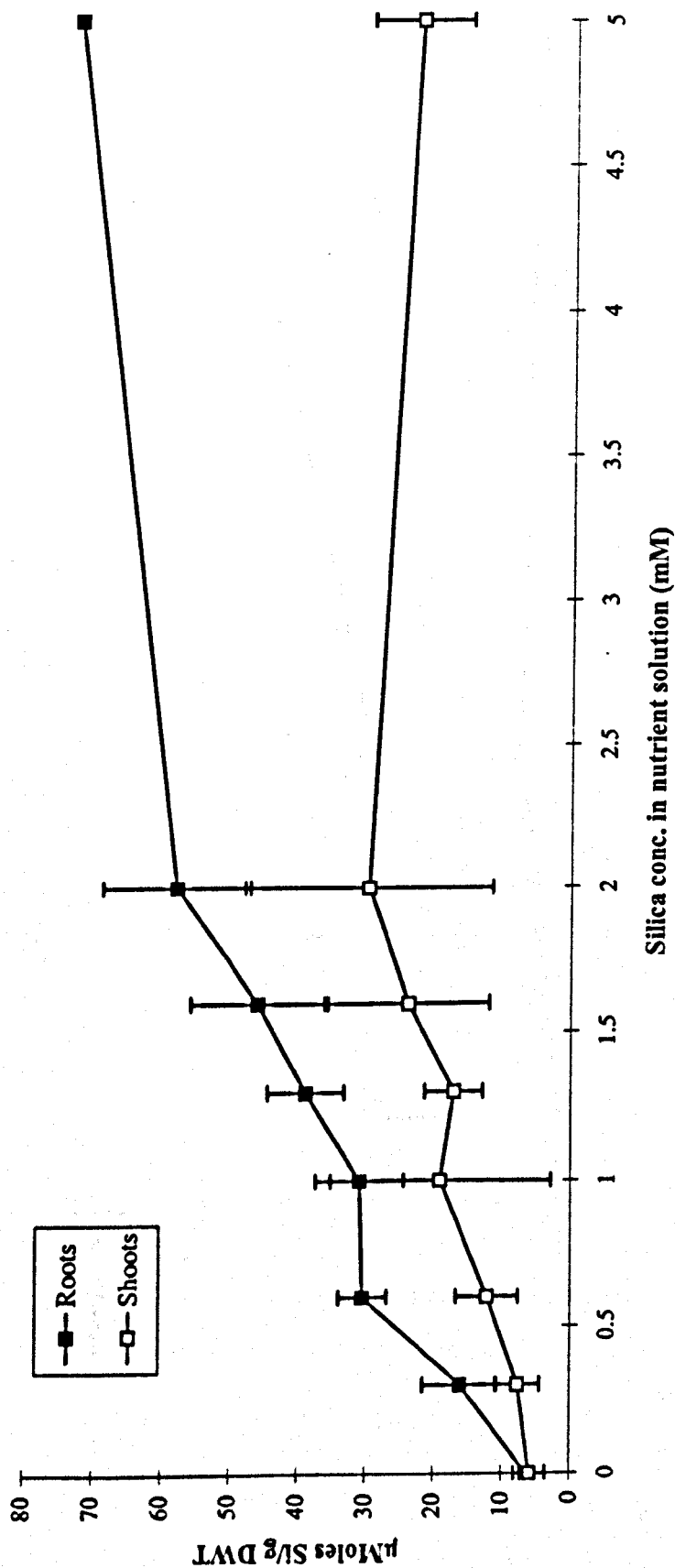
When wheat plants were exposed to increasing silica concentration, the content of silica in the roots and shoots increased (fig. 5.1). The Si content of the roots and shoots of plants not exposed to silica were 6.44 and 5.88  $\mu\text{moles/gDWT}$  respectively. These values rose steadily to 57.79  $\mu\text{moles/gDWT}$  for roots and 29.68  $\mu\text{moles/gDWT}$  for shoots when the plants were exposed to 2 mM silica solution. The rate of increase was greater in the roots than in the shoots. There appears to be a decrease in this rate when the silica concentration exceeds 2 mM in both the roots and the shoots. The silica content of the roots and shoots was 72.3 and 22.4  $\mu\text{moles/gDWT}$  respectively when exposed to 5 mM silica.

#### 5.3.2. 24 Hour Time Course of Silica Uptake.

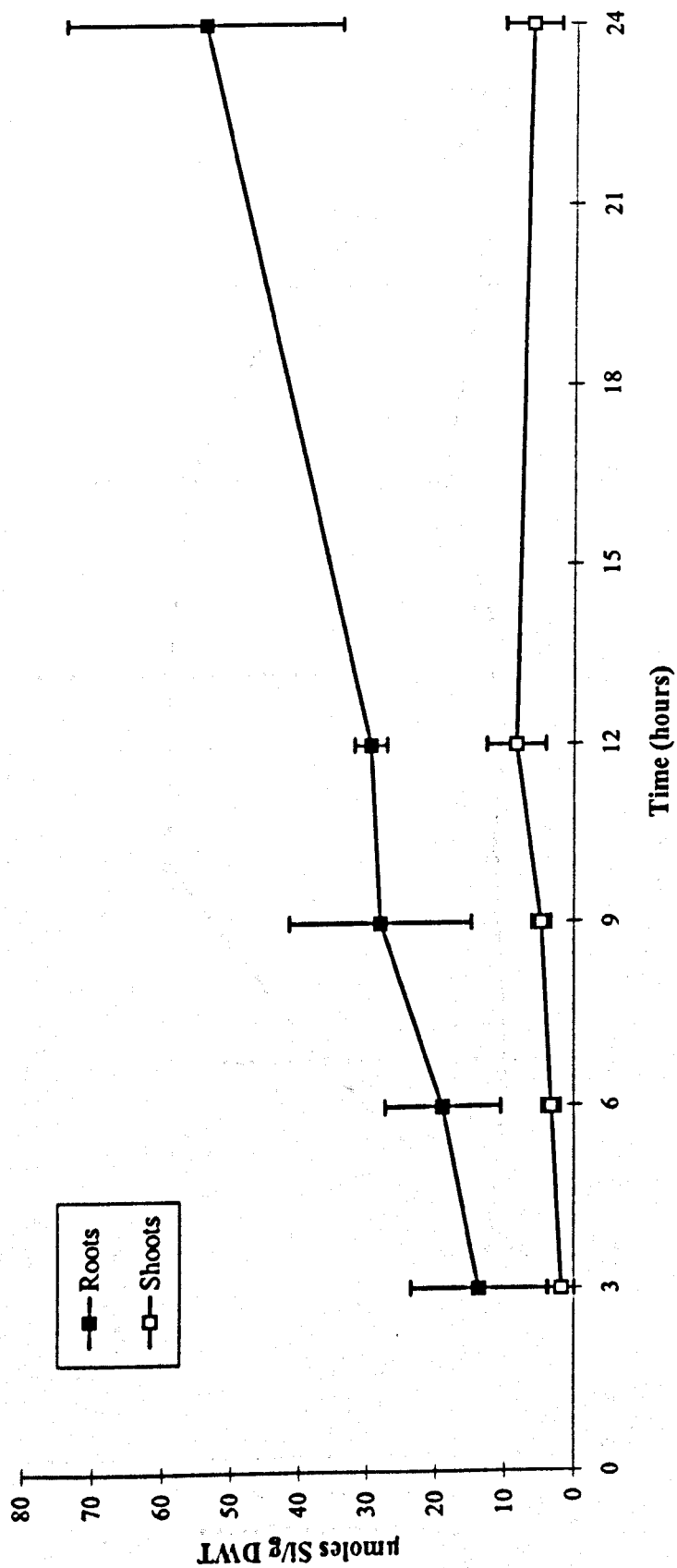
With increased exposure to 2 mM silica the content of silica within the roots and shoots of the wheat plants increased (fig. 5.2). In roots, this increase appeared to be constant up to 24 h. exposure. After 3 h. exposure the roots contained greater amounts of silica than the shoots (13.76  $\mu\text{moles/gDWT}$  compared to 1.85  $\mu\text{moles/gDWT}$ ). Also the rate of silica accumulation is greater in the roots than the shoots over the 24 h. period. After this period the roots contained 53.88  $\mu\text{molesSi/gDWT}$  compared to the shoots, which contained 6.23  $\mu\text{molesSi/gDWT}$ . The amount of silica in the shoot did not increase significantly over the time period 3-24 h. (d.f.=2,  $p=0.05$ ,  $t=-1.40$ ).

#### 5.3.3. The Effect of pH on Silica Uptake.

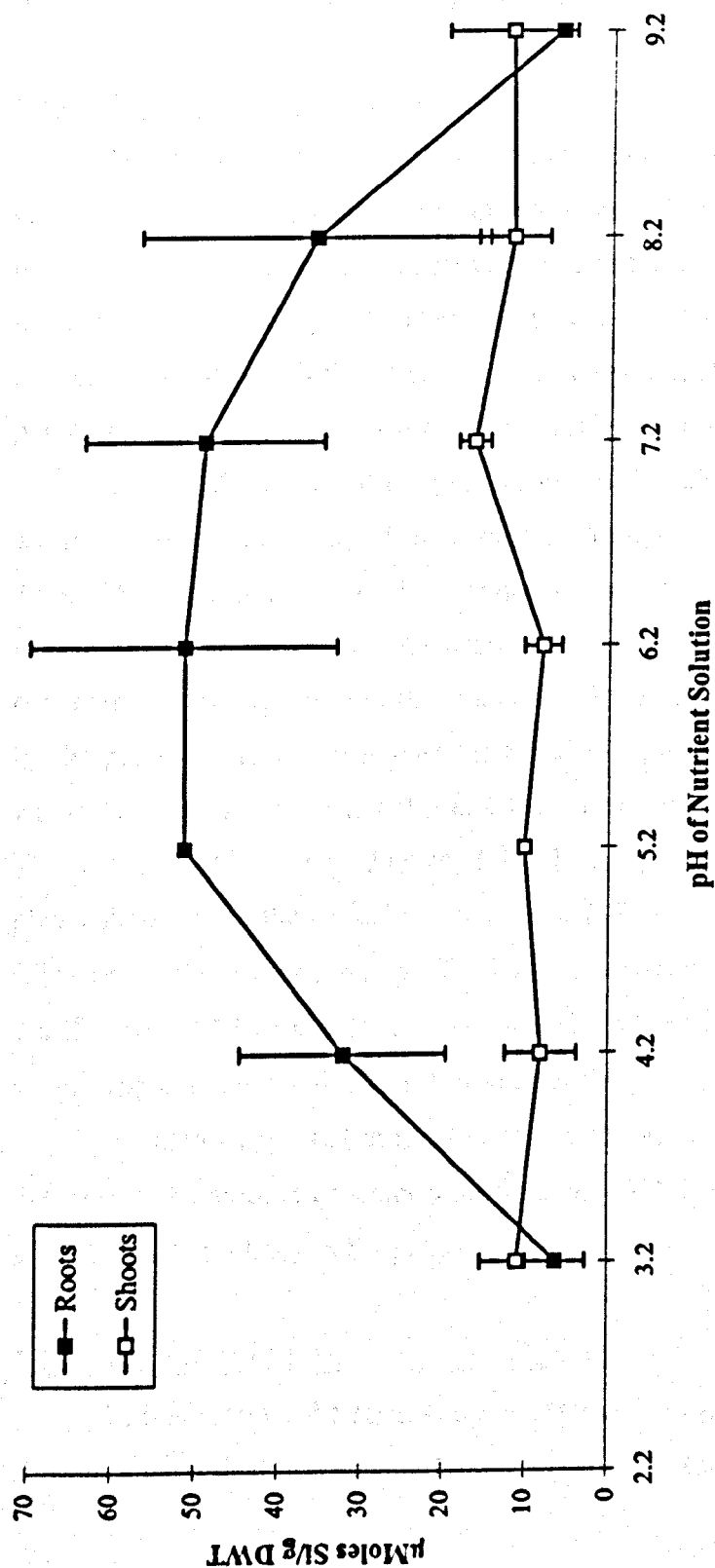
The roots and shoots showed contrasting responses to the changes of pH of the nutrient solution, with respect to their silica content (fig. 5.3). The shoot silica content appeared to be relatively unaffected by the pH of the nutrient solution. There was a slight increase in silica content with increasing pH, raising from 10.97  $\mu\text{moles/gDWT}$  to 12.2  $\mu\text{moles/gDWT}$  over the pH range 3.2 - 9.2.



**Figure 5.1.** The effect of increasing silica concentration within the nutrient solution on the silica content of the roots and shoots of wheat plants. The plants were grown in the solution for 24 hours (each point represents the mean average of 2 replicates  $\pm$  std.).



**Figure 5.2.** The quantity of silica within wheat plants grown in nutrient solution containing 2 mM silica over a 24 hour time period (each point represents the mean average of 2 replicates  $\pm$  std. for root data. For shoot data each point represents the mean average of 3 replicates  $\pm$  std.).



**Figure 5.3.** The effect of pH of the nutrient solution on the silica content of wheat plants. The nutrient solution contained 2 mM silica and the plants were grown for 24 hours in the solution (each point represents the mean average of 2 replicates  $\pm$  std.).



The silica content of the roots appeared to be greatly affected by the pH of the nutrient solution. There is a broad peak, over the pH range 5.2 - 7.2, where silica content is high (about 50  $\mu\text{molesSi/gDWT}$ ). There was a rapid decrease before and after this region, falling to 6.17  $\mu\text{moles/gDWT}$  at pH 9.2 and 6.3  $\mu\text{moles/gDWT}$  at pH 3.2.

#### 5.3.4. Transpiration Experiment.

When wheat plants were exposed to 2 mM Si solution for 24 hours, chemical analysis of 25 Si+ plants shows relatively little difference between the Si content in the roots and shoots [2.362 and 2.267  $\mu\text{moles}$  per 25 plants respectively (tab. 5.2a)]. The chemical analysis of the grains of both Si- and Si+ showed very little difference in Si content (2.138 and 2.071  $\mu\text{moles}$  per 25 plants). Both contained high levels of silica (tab. 5.2a).

Chemical analysis of the plants showed that 25 Si+ plants had accumulated an additional 4.186  $\mu\text{moles Si}$  in total during the experiment. These 25 plants used 1.609 ml water during that time (calculated from the overall change in weight of the culture tube, tab. 5.2b). As the concentration of silica within the solution and the amount of water "used" by the plants are known it is possible to calculate the quantity of silica that would have entered the plant if it had been acquired by mass flow alone. This is the TSFC (see introduction, 5.1). In the present investigation the 25 plants should have theoretically contained 3.217  $\mu\text{moles silica}$  (tab. 5.2b), if silica enters the plant passively. They actually contained 4.186  $\mu\text{moles Si}$ , a difference of 0.969  $\mu\text{moles Si}$ . The TSFC was calculated to be 1.3, thus suggesting that some silica is acquired actively.

Si- plants appeared to transpire a greater amount of water than the Si+ plants, the amount of water transpired per gFWT was significantly greater ( $n=25$ ,  $p=0.10$ ) in Si- plants.

#### 5.3.5. The Effect of Inhibitors on Ion Content.

In this section, the results, when expressed as  $\mu\text{moles/gDWT}$ , showed a great deal of variation between replicate experiments, and this

**Table 5.2.** The actual and calculated silica content of plants grown for 24 hours in nutrient solution containing 2 mM silica based upon transpiration rates.

**Table 5.2a.** Silica Content Measured in 25 Wheat Plants.

Plant Organ	Si +		Si -	
	Dry Weight (g)	μmoles Si	Dry Weight (g)	μmoles Si
<b>Root</b>	0.079	2.362	0.0806	0.225
<b>Grain</b>	0.8511	2.071	0.8911	2.138
<b>Shoot</b>	0.0898	2.267	0.0879	0.151
<b>Total</b>	1.0206	6.700	1.0596	2.514

**Table 5.2b.** Silica Estimated to be in 25 Wheat Plants.

The estimated amount of silica within the plants is calculated by multiplying the amount of water used by the concentration of silica within the nutrient solution, which was 2 mM Si.

All Values are based upon 25 plants.		Weight loss (ml H <sub>2</sub> O)	Water Used By Plants (ml)	Si in Plant.(μmoles) Calculated.
<b>Si -</b>	Without Plants	2.4105.		
	With Plants	5.7628	3.3523	0
<b>Si +</b>	Without Plants	2.2800		
	With Plants	3.8886	1.6086	3.2172

meant that the results were not strong enough to be subjected to t-test statistics. Thus, it was considered that the results were more suitably expressed as a percentage of control plants, i.e. plants which were grown in identical nutrient solution at the same time as the experimental plants, but without the inhibitor present (alcohol concentrations were also identical where necessary, see methods, section 5.2.6), for each individual replicate experiment. The mean of the calculated percentages of the control plants of the 4 replicate experiments are displayed graphically. When  $\mu\text{moles/gDWT}$  data (presented in appendix V, tab. 1-5) was significant this is referred to in the text.

Total potassium and phosphate content was measured, as opposed to measuring quantity taken up during the experiment, for several reasons. The young wheat seedlings showed great variability and therefore the quantity of elements within the plant analyzed after germination may not compare with other individual plants analyzed after the experiment. Also, total content does give an indication of ion uptake, whilst before and after measurements would only exaggerate the results obtained. Additionally, potassium and phosphate data was obtained as an indicator of inhibitor effect and exact measurement of uptake and transport was not necessary.

#### 5.3.5a. The Effect of Sodium Orthovanadate on the Ion Content of Wheat Plants.

##### **Silica**

Sodium orthovanadate (vanadate) appeared to stimulate silica uptake by wheat plants exposed to 25 and 50  $\mu\text{M}$  vanadate for 24 hours (fig. 5.4a), when measured as a percentage of control plants, (i.e. plants which had been exposed to the same silica concentration but not to the inhibitor). Both the root and shoot showed an increased quantity of silica after exposure to 50  $\mu\text{M}$  vanadate (122.3% and 140.5% respectively).

However, the shoot silica content was reduced to 79.4% of control plants when exposed to 75  $\mu\text{M}$  vanadate. This was further reduced, to 69.5% of control plants, when exposed to 100  $\mu\text{M}$  vanadate.

**Figure 5.4.** The effect of sodium orthovanadate on the silica, potassium and phosphate content of wheat plants grown in nutrient solution for 24 hours.

**Figure 5.4a.**

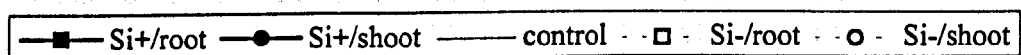
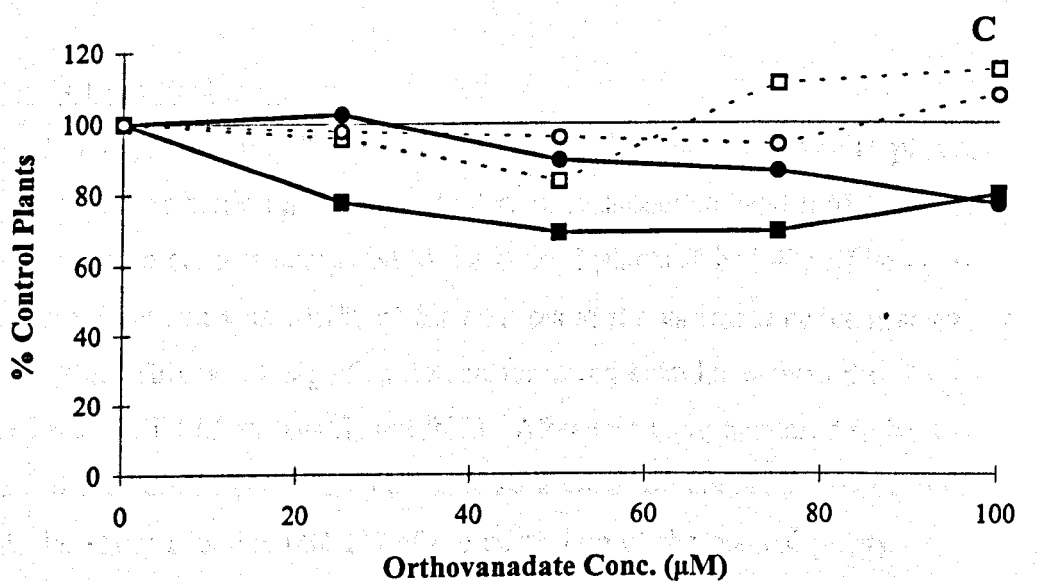
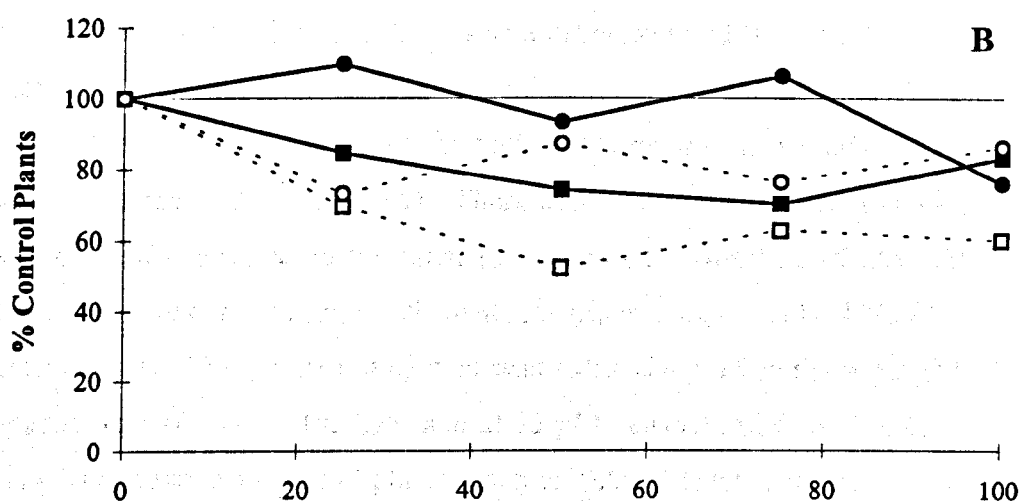
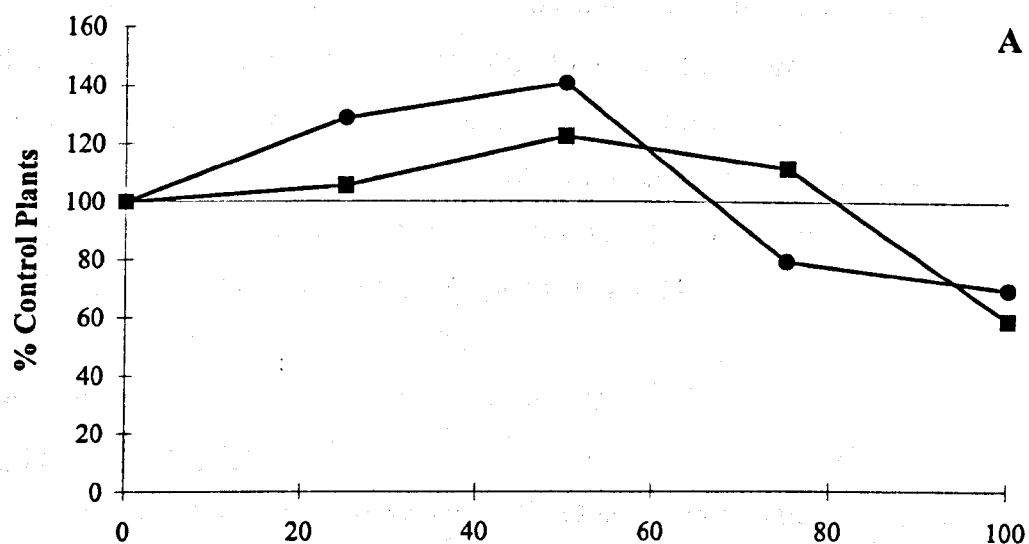
The effect of sodium orthovanadate on the silica content of wheat plants grown in nutrient solution for 24 hours. The nutrient solution contained 2 mM silica. The control plants were grown in identical nutrient solutions minus the sodium orthovanadate. Mean average of 4 replicates.

**Figure 5.4b.**

The effect of sodium orthovanadate on the potassium content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the sodium orthovanadate. Mean average of 4 replicates.

**Figure 5.4c.**

The effect of sodium orthovanadate on the phosphate content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the sodium orthovanadate. Mean average of 4 replicates.



The roots contained 111.74% of the silica in control plants when the plants were exposed to 75  $\mu$ M vanadate, in contrast to the decrease observed in the shoots. The root silica content was reduced greatly to 58.95% of control plants when the vanadate concentration was increased to 100  $\mu$ M. This result was significant when calculated from the  $\mu$ moles/gDWT data obtained (d.f.=6, p=0.1, t=1.901).

## **Potassium.**

### **Results from Si+ Plants.**

Vanadate seemed to have very little effect on potassium content of the shoots of the plants (fig. 5.4b). When the vanadate was at the highest concentration tested, 100  $\mu$ M, there was a decrease to 75.8% of control plants.

The potassium content of the root decreased with increasing vanadate concentration (fig. 5.4b). There appeared to be a linear decrease to 70.01% of control when the vanadate concentration reached 75  $\mu$ M. At 100  $\mu$ M vanadate concentration there was a slight increase to 83.12% of the control plants. The potassium content was reduced significantly at 50  $\mu$ M vanadate (d.f.=6, p=0.1, t=2.298) and at 75  $\mu$ M vanadate (d.f.=6, p=0.05, t=2.493) compared to control plants when calculated from  $\mu$ moles potassium/gDWT data.

### **Results from Si- Plants.**

The general trends are similar to those obtained from the Si+ plants, although the reduction is slightly greater. The shoots showed a decrease in the potassium content compared to the control plants (fig. 5.4b). The potassium content was 73.1% of the controls at the vanadate concentration of 25  $\mu$ M. This was a significant decrease when calculated from the  $\mu$ moles/gDWT (d.f.=6, p=0.1, t=1.967). After this there appeared to be a gradual increase in potassium content. At a vanadate concentration of 100  $\mu$ M, the shoot contained 86.1% of the potassium of the control plants.

The roots showed a greater decrease in potassium content than the

shoots (fig. 5.4b). Potassium content of the roots was reduced to 52.16% of the control plants when the vanadate concentration equalled 50  $\mu\text{M}$ . This increased to 62.2% when the concentration was increased to 75  $\mu\text{M}$ . At 100  $\mu\text{M}$  vanadate the potassium content was 59.9% of the control plants.

## **Phosphate**

### **Results From Si+ Plants.**

The phosphate content of the shoots was relatively unaffected by exposing the plants to 25  $\mu\text{M}$  vanadate solution (fig. 5.4c). However, the phosphate content decreased gradually with increasing vanadate concentration, to 77.1% at 100  $\mu\text{M}$  vanadate. Results obtained from exposing the plants to 75 and 100  $\mu\text{M}$  vanadate showed a significant ( $t=4.84$  and  $t=3.604$  respectively  $p=0.05$ ,  $d.f.=6$ ) decrease in phosphate content, when expressed as  $\mu\text{moles phosphate/gDWT}$ .

The phosphate content of the roots also showed a decrease compared to the control plants (fig. 5.4c). At 25  $\mu\text{M}$  vanadate the phosphate content was reduced to 77.9% of the control plants. This decreased further to 69.3% when the plants were exposed to 75  $\mu\text{M}$  vanadate. However, this increased to 79.8% when the vanadate concentration was increased to 100  $\mu\text{M}$ . When the results were expressed as  $\mu\text{moles phosphate/gDWT}$ , the decrease at 50  $\mu\text{M}$  was significant ( $d.f.=6$ ,  $p=0.1$ ,  $t=2.298$ ) as was the decrease at 75  $\mu\text{M}$  ( $d.f.=6$ ,  $p=0.05$ ,  $t=9.331$ ).

### **Results from Si- Plants.**

Orthovanadate did not greatly affect the phosphate content of the Si- plants (fig. 5.4c). Shoots only showed a slight decrease in phosphate content compared to the control plants. The greatest decrease was to 93.9% of the control when the plants were exposed to 75  $\mu\text{M}$  vanadate. When exposed to 100  $\mu\text{M}$  vanadate there was a slight increase in phosphate content, up to 108% of control.

The phosphate content of the roots only showed a slight decrease when exposed to 25  $\mu\text{M}$ , down to 95.8% of control. This decreased further

to 83.6% when the concentration of vanadate was increased to 50  $\mu\text{M}$ . However, the phosphate content increased to 111.3% of the control plants and again to 115.4% over the vanadate concentrations of 75  $\mu\text{M}$  and 100  $\mu\text{M}$  respectively.

### SUMMARY.

Sodium orthovanadate stimulated silica uptake when the concentration of vanadate was lower than 50  $\mu\text{M}$ . When the vanadate concentration was increased there was a decrease in silica content of the plant. Generally, over the concentration range 0 - 100  $\mu\text{M}$ , vanadate caused a decrease in potassium content of both Si<sup>+</sup> and Si<sup>-</sup> plants. Vanadate had little effect on the phosphate content of Si<sup>-</sup> plants. Si<sup>+</sup> plants tended to show a greater decrease in phosphate when exposed to vanadate.

#### 5.3.5b. The Effect of Erythrosin B on the Ion Content of Wheat Plants.

##### Silica

Erythrosin B (EB), in the concentration range 0 - 10  $\mu\text{M}$ , generally caused a decrease in the silica content of the shoot of plants exposed for 24 hours (fig. 5.5a). When the plants were subjected to 0.5  $\mu\text{M}$  EB the quantity of silica within the shoot was reduced to 59.3% of the control plants. There was then a slight increase to 71.0% of the silica within the control plants when the EB concentration was elevated to 1.0  $\mu\text{M}$ . However, the general declining trend of silica content continued when the plants were exposed to 5  $\mu\text{M}$ . This concentration produced a decrease to 58.5% silica of the control plants. The lowest silica content in the plants was recorded when the plants were subjected to 10  $\mu\text{M}$  EB, the quantity of silica within the shoots of the experimental plants was reduced to 44.15 % of the control plants. The results obtained using 5 and 10  $\mu\text{M}$  EB produced results which, when expressed as  $\mu\text{moles silica/gDWT}$ , were significantly different from the control plants (5  $\mu\text{M}$ : d.f.=6,  $p=0.05$ ,  $t=2.605$ ; 10  $\mu\text{M}$ : d.f.=6,  $p=0.05$ ,  $t=3.550$ ).

When exposed to low concentrations of EB, in the range 0 - 5  $\mu\text{M}$ ,



**Figure 5.5.** The effect of erythrosin B on the silica, potassium and phosphate content of wheat plants grown in nutrient solution for 24 hours.

**Figure 5.5a.**

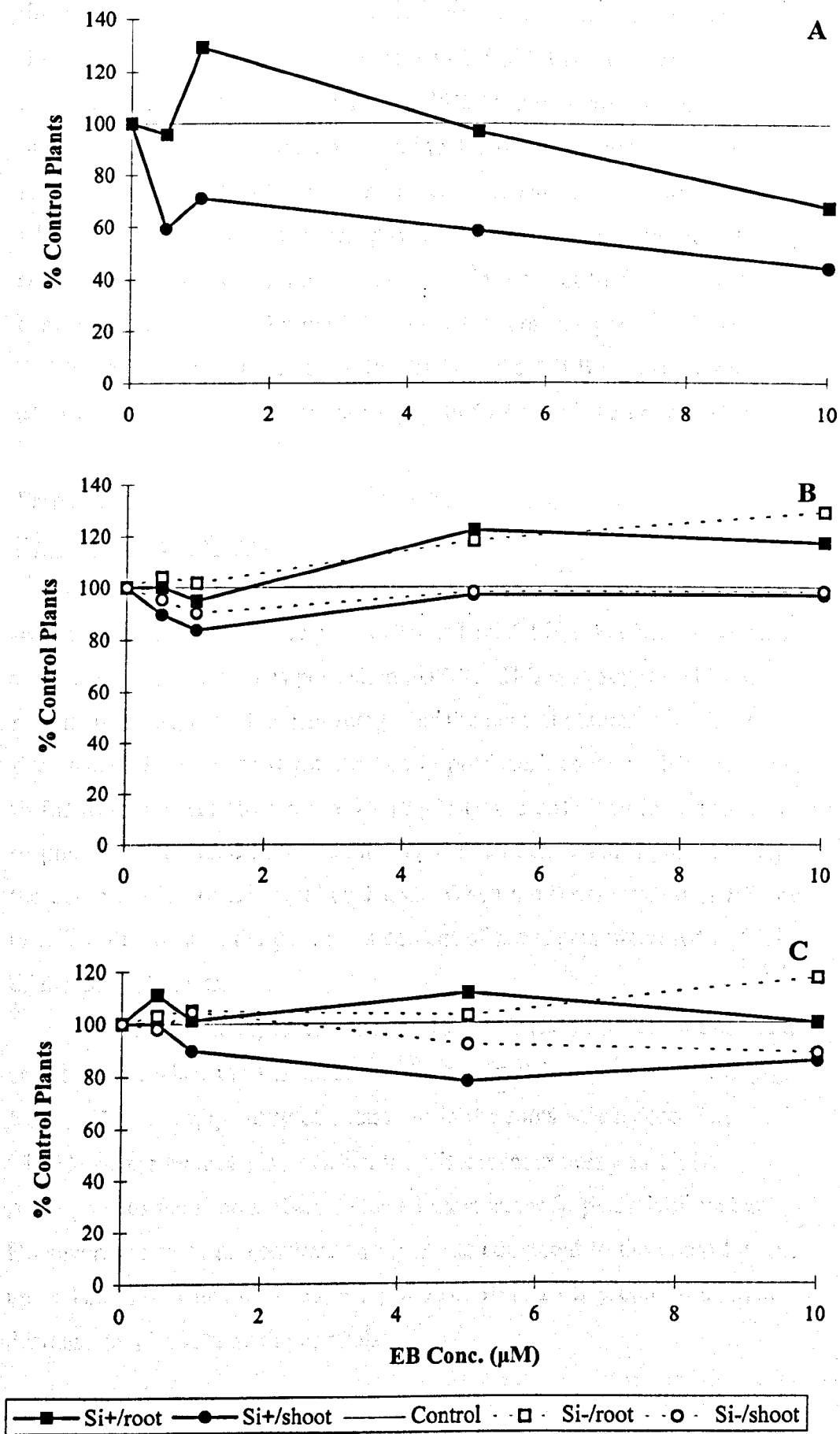
The effect of erythrosin B on the silica content of wheat plants grown in nutrient solution for 24 hours. The nutrient solution contained 2 mM silica. The control plants were grown in identical nutrient solutions minus the erythrosin B. Mean average of 4 replicates.

**Figure 5.5b.**

The effect of erythrosin B on the potassium content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the erythrosin B. Mean average of 4 replicates.

**Figure 5.5c.**

The effect of erythrosin B on the phosphate content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the erythrosin B. Mean average of 4 replicates.



the roots of the plants did not display a similar decrease in silica content as the shoots (fig. 5.5a). When subjected to 0.5  $\mu\text{M}$  EB there was only a negligible drop in silica content, to 95.75% of the control plants. However, when the EB concentration was raised to 1  $\mu\text{M}$ , the quantity of silica increased to 129.30% of the controls. This increase was not significant when expressed as  $\mu\text{moles silica/gDWT}$ . Further increases in the EB concentration caused the silica content within the root to decline. EB concentration of 5  $\mu\text{M}$  did not affect the silica content greatly, it was 97.04% of the control plants. A further increase in EB concentration to 10  $\mu\text{M}$  caused the quantity of silica to drop to 67.44% of the control plants.

### **Potassium.**

#### **Results from Si+ Plants.**

EB did not produce any significant effect on the potassium content of the roots or shoots of the plants exposed for 24 hours, when the results were calculated as  $\mu\text{moles potassium/gDWT}$ . This was supported by the results when expressed as percentages of the control plants. The shoot demonstrated only very slight changes in potassium content when subjected to EB in to concentration range 0 - 10  $\mu\text{M}$  (fig. 5.5b). The lowest quantity of potassium recorded in the shoot was 83.9% of the control plants, when the concentration of EB equalled 1  $\mu\text{M}$ . When the concentration of EB was raised, up to 10  $\mu\text{M}$ , the potassium content of the shoots increased to 97.1% of the control plants.

The roots displayed a similar decreasing trend in potassium content, as the shoots when the EB concentration was in the range of 0 -1  $\mu\text{M}$  (fig. 5.5b). The lowest quantity of potassium in the roots was recorded as 93.05% of the control plants, when the EB concentration was 1  $\mu\text{M}$ . Analogous to the shoots, there followed an increase in potassium content. However, the roots demonstrated an increase compared to the control plants, up to 122.17%, when exposed to 5  $\mu\text{M}$  EB. 10  $\mu\text{M}$  EB produced a slight decrease to 117.02% of the controls.

### Results from Si- Plants.

Si- plants displayed similar results to the Si+ plants (fig. 5.5b). The lowest potassium content of the shoots was recorded when the plant were subjected to 1  $\mu\text{M}$ , it was reduced to 90.3% of the control plants. This increased to 98.4% of the controls when the plants were subjected to 10  $\mu\text{M}$ .

The roots from Si- plants also displayed comparable results with roots from Si+ plants, although the potassium content was not reduced to below that of the control plants (fig. 5.5b). The highest quantity of potassium measured was 118.38% of the control plants when the concentration of EB was 10  $\mu\text{M}$ .

### **Phosphate.**

#### Results from Si+ Plants.

EB had a slightly greater effect on phosphate content than on potassium content. 0.5  $\mu\text{M}$  EB produced no change in the quantity of phosphate within the shoots of the plants (fig. 5.5c). Increasing the EB concentration to 1  $\mu\text{M}$  caused a decline in the quantity of phosphate to 89.6% of the control. Raising the concentration of EB caused a decrease in phosphate, such that 10  $\mu\text{M}$  EB resulted in the phosphate content being reduced to 85.5% of the control. This result was significantly different from the control when expressed as  $\mu\text{moles phosphate/gDWT}$  (d.f.=6,  $p=0.05$ ,  $t=3.015$ ).

The roots showed variable phosphate content when exposed to EB concentrations in the range 0 - 10  $\mu\text{M}$  (fig. 5.5c). The quantity of phosphate was highest when the EB concentration equalled 0.5  $\mu\text{M}$  and 5  $\mu\text{M}$ , when it was about 112% of the control plants. The other concentrations of EB produced virtually no change to the phosphate content of the roots of the plants.

#### Results from Si- Plants.

Similar trends were observed as those recorded for Si+ plants. For

the shoots, the lowest phosphate content measured was 88.4% for the control plants when subjected to 10  $\mu\text{M}$  EB (fig. 5.5c).

The roots, similarly, did not display a great change in phosphate content when exposed to EB (fig. 5.5c). There was a gradual increase in phosphate, up to 118.38%, when the plants were subjected to 10  $\mu\text{M}$ .

### SUMMARY.

EB appeared to affect the silica content differently at high and low concentrations. Under 1  $\mu\text{M}$ , EB appeared to increase the silica content within the root and decrease the silica content within the shoot. When the EB concentration was increased there was an overall decline in the silica content of the plant. There was very little effect on the potassium content, although at high concentrations there was an increase in the quantity of potassium within the roots. There was little trend to the affect of EB on the phosphate content of the wheat plants.

#### 5.3.5c. The Effect of Diethylstilbestrol on the Ion Content of Wheat Plants. Silica.

Diethylstilbestrol (DES) clearly affects the silica content of wheat plants exposed to concentrations in the range 0 - 100  $\mu\text{M}$  for 24 hours (fig. 5.6a). The quantity of silica within the shoot is decreased when the plant was subjected to 25  $\mu\text{M}$  DES. The shoot contained 78.9% of the silica contained within the control plants. This was a significant decrease when calculated from  $\mu\text{moles silica/gDWT}$  data (d.f.=6,  $p=0.1$ ,  $t=2.060$ ). The silica content of the shoots increased slightly to 81.89% of the control plants when the DES concentration was raised to 50  $\mu\text{M}$ . This was not significantly different from the control plants. Increasing the DES concentration up to 75  $\mu\text{M}$  did not have any further affect on the quantity of silica present in the shoot. However when the DES concentration was elevated to 100  $\mu\text{M}$ , the silica content dropped to 38.96% of the control plants. Again, this was a significant reduction compared to the control plants (d.f.=6,  $p=0.05$ ,  $t=4.245$ ).

**Figure 5.6.** The effect of DES on the silica, potassium and phosphate content of wheat plants grown in nutrient solution for 24 hours.

**Figure 5.6a.**

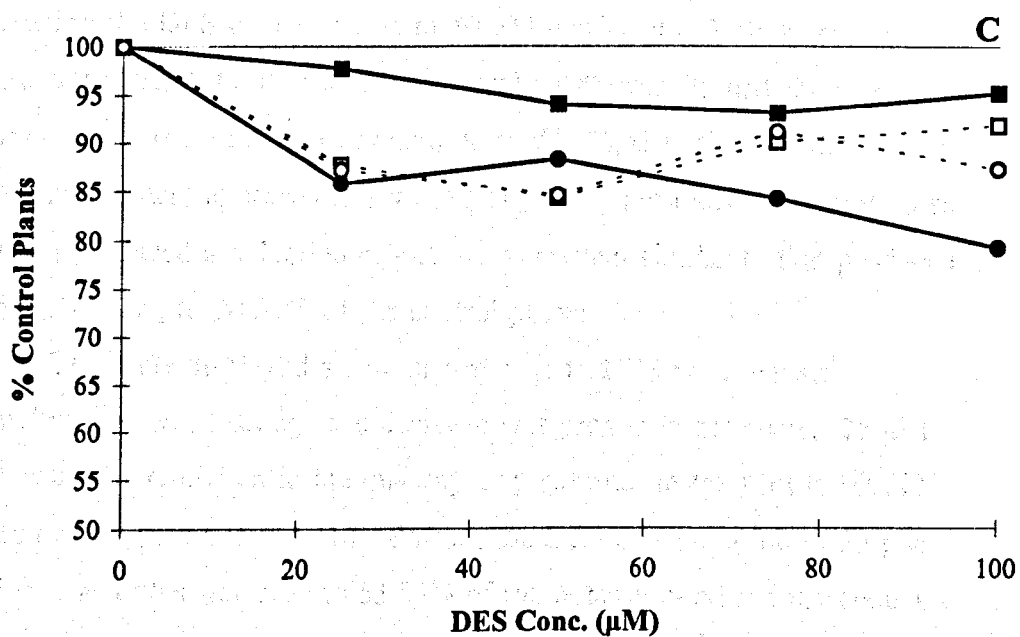
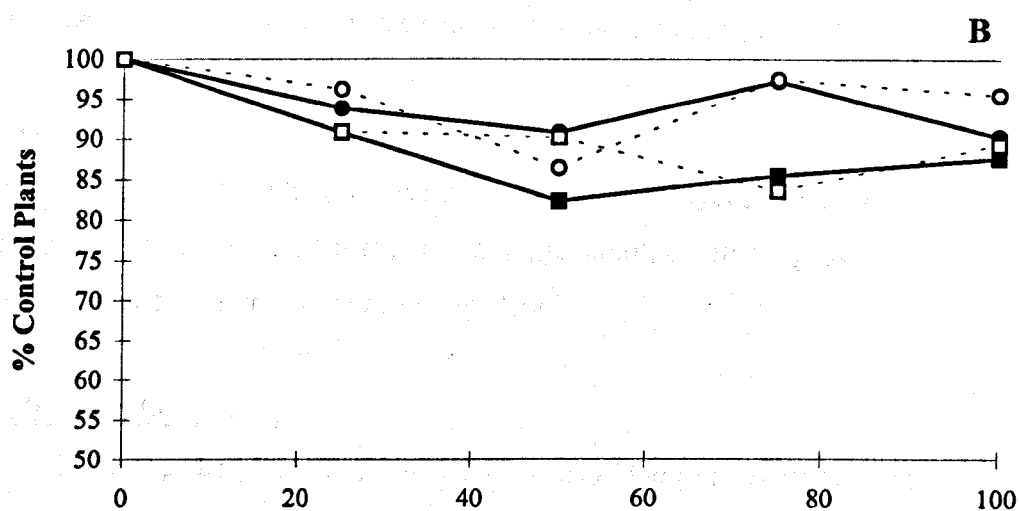
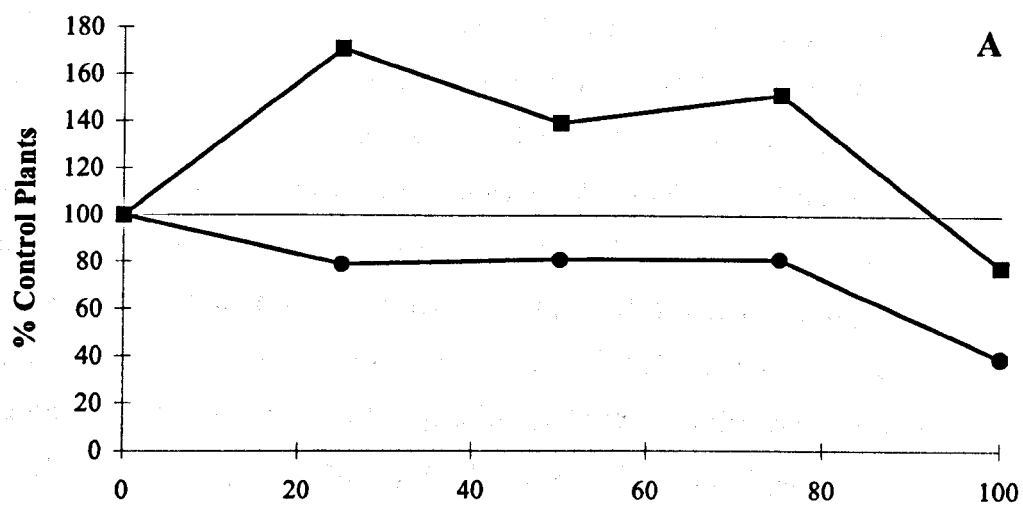
The effect of DES on the silica content of wheat plants grown in nutrient solution for 24 hours. The nutrient solution contained 2 mM silica. The control plants were grown in identical nutrient solutions minus the DES. Mean average of 4 replicates.

**Figure 5.6b.**

The effect of DES on the potassium content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the DES. Mean average of 4 replicates.

**Figure 5.6c.**

The effect of DES on the phosphate content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the DES. Mean average of 4 replicates.



—■— Si+/root    —●— Si+/shoot    — Control    - - □ - Si-/root    - - ○ - Si-/shoot

The roots of plants exposed to DES displayed an increase in silica content compared to control plants, over the concentration range 0 - 75  $\mu\text{M}$  (fig. 5.6a). When subjected to DES concentration of 25  $\mu\text{M}$ , the silica content rose to 169.32% of the control plants. This was a significant increase when expressed as  $\mu\text{moles silica/gDWT}$  (d.f.=6,  $p=0.05$ ,  $t=3.187$ ). Despite the fact that the silica content fell to 141.07% of the control when the DES concentration was increased to 50  $\mu\text{M}$ , this was still a significant increase in comparison to the controls (d.f.=6,  $p=0.05$ ,  $t=4.950$ ). The quantity of silica was not markedly affected when the DES concentration was elevated to 75  $\mu\text{M}$ , resulting in 153.98% of the control plants. However, when the DES concentration was further increased to 100  $\mu\text{M}$ , the silica content in the root decreased to 80.59% of the control.

### **Potassium.**

DES, over the whole concentration range employed, caused a decline in potassium content, of both the root and the shoot, of plants grown in either Si- and Si+ nutrient solution (fig. 5.6b).

### **Results from Si+ Plants.**

Exposing the plants to 25  $\mu\text{M}$  DES caused the potassium content of the shoots to be reduced to 93.9% of the control plants (fig. 5.6b). Increasing the DES concentration to 50  $\mu\text{M}$  produced a further decline in potassium content, to 90.0% of the control. Although 75  $\mu\text{M}$  DES caused an increase in the quantity of potassium to 97.3%, the effect on the potassium content appeared to level off as raising the DES concentration to 100  $\mu\text{M}$  produced a reduction of potassium content similar to that produced by 50  $\mu\text{M}$  DES, to 90.36% of the control plants.

The roots displayed a similar response to DES with respect to potassium content, although the decrease was greater in the roots. 25  $\mu\text{M}$  DES caused a reduction in the quantity of potassium in the root to 90.71% of the control plants (fig. 5.6b). Raising the DES concentration to 50  $\mu\text{M}$  produced a further decrease to 83.87% of the potassium within the control



plants. Comparable with the shoots, further increases in DES concentration only had slight effect on the potassium content of the roots. The potassium content was reduced to 89.69% of the control plants.

#### Results From Si- Plants.

The results acquired using Si- plants were analogous to those obtained from Si+ plants (fig. 5.6b). 25  $\mu$ M DES caused the potassium content of the shoots to be reduced to 96.23% of the control plants. A further decline in the quantity of potassium, to 86.44%, was observed when the DES concentration was increased to 50  $\mu$ M. The potassium content was only slightly affected by higher concentrations of DES, such that 100  $\mu$ M caused a small reduction to 95.61% of the control plants.

The roots also demonstrated a decline in potassium content with increasing DES concentration (fig. 5.6b). When exposed to 25  $\mu$ M DES the quantity of potassium was reduced to 91.22% of the control plants. This continued, such that when the plants were subjected to 75  $\mu$ M DES, the potassium content had decreased to 83.70% of the controls. This was a significant decrease when expressed as  $\mu$ moles potassium/gDWT (d.f.=6,  $p=0.05$ ,  $t=3.309$ ). There was a slight increase, up to 89.22%, when the DES concentration was elevated to 100  $\mu$ M.

#### **Phosphate.**

The alteration of phosphate content of wheat plants exposed to DES was similar to the changes recorded when measuring potassium content. DES, over the concentration range utilized in this investigation, produced a reduction in phosphate content in Si+ and Si- plants, both within the shoots and the roots (fig. 5.6c).

#### Results From Si+ Plants.

Both shoots and roots display a reduction in phosphate content with increasing DES concentration, although the reduction in the shoots was greater than the reduction observed in the roots (fig. 5.6c). The quantity of

phosphate within the shoot decreased to 85.8% of the control plants when exposed to 25  $\mu\text{M}$  DES. Further increases in DES produced a gradual decline in phosphate content, such that 100  $\mu\text{M}$  DES caused a reduction in the quantity of phosphate to 79.07% of the control plants. This was a significant decrease when calculated from  $\mu\text{moles phosphate/gDWT}$  (d.f.=6,  $p=0.05$ ,  $t=2.634$ ).

The roots were less affected by DES in the nutrient solution than the shoots. A DES concentration of 25  $\mu\text{M}$  caused a decrease to 97.48% of the control plants (fig. 5.6c). Again, increasing the DES concentration produced a gradual decrease in phosphate content. The quantity of phosphate within the roots was reduced only slightly, to 95.13% of the controls, when the concentration of DES equalled 100  $\mu\text{M}$ .

#### Results From Si- Plants.

These results displayed similar trends to those observed for Si+ plants, except that the phosphate content in both the shoot and the root were similarly affected by the DES concentration (fig. 5.6c). When subjected to 25  $\mu\text{M}$  DES the phosphate content of the shoot decreased to 87.18% of the control plants, this is very similar to the result obtained with Si+ plants (85.8% of control). This was followed by a further reduction to 84.49% of control when exposed to 50  $\mu\text{M}$  DES. There followed a slight increase in phosphate content when the DES concentration was raised. DES concentration of 75  $\mu\text{M}$  caused an increase to 91.07% of control, although when DES concentration equalled 100  $\mu\text{M}$  the phosphate content was 87.19% of the control plants.

The results acquired from the roots were analogous to those obtained from the shoots. 25  $\mu\text{M}$  DES caused a reduction of phosphate to 89.05% of the control plants (fig. 5.6c). Increasing the DES concentration further to 50  $\mu\text{M}$  produced a subsequent decrease in phosphate content to 86.45% of control. However, additional increases in DES concentration induced a rise in phosphate content. When subjected to 100  $\mu\text{M}$  DES, the quantity of phosphate in the roots was 93.04% of the control plants.

## SUMMARY.

DES increased silica content within the roots and decreased the silica content in the shoots, until the DES concentration reached 100  $\mu\text{M}$ . Then there was an overall decrease in silica content. Generally DES caused a reduction in the quantity of potassium within the wheat plants. This was also observed when measuring phosphate content of the wheat plants exposed to DES.

### 5.3.5d. The Effect of Nigericin on the Ion Content of Wheat Plants.

#### **Silica.**

Nigericin, at a concentration of 0.3  $\mu\text{M}$ , appeared to stimulate silica transport to the shoots of the wheat plants when exposed to 2 mM silica solution (fig. 5.7a). The plants contained 144.2% of the silica contained in control plants which had not been exposed to the inhibitor solution. When the concentration of the nigericin was increased the silica content was reduced to 92.5% of the control plants. Following this, the silica content continued to decrease with increasing nigericin concentration. When the nigericin concentration equalled 10  $\mu\text{M}$  (data not presented graphically) the silica content was reduced to 61.4% of the control plants.

The silica content of the roots of the plants exposed to 0.3  $\mu\text{M}$  nigericin was decreased to 57.6% of the control plants (fig. 5.7a). This decrease was significant when expressed as  $\mu\text{moles silica/gDWT}$  (d.f.=6,  $p=0.05$ ,  $t=2.842$ ). There was a slight increase in silica content when exposed to 0.6  $\mu\text{M}$  nigericin, up to 78.40% of control plants. The lowest silica content occurred after the plant had been exposed to 1  $\mu\text{M}$  nigericin, when the silica content was 54.46% of control. Again, this was a significant decrease when compared to the control plants (d.f.=6,  $p=0.05$ ,  $t=2.530$ ). There was a slight increase in silica content when using 10  $\mu\text{M}$  nigericin (data not presented graphically), up to 67.77%.

**Figure 5.7.** The effect of nigericin on the silica, potassium and phosphate content of wheat plants grown in nutrient solution for 24 hours.

**Figure 5.7a.**

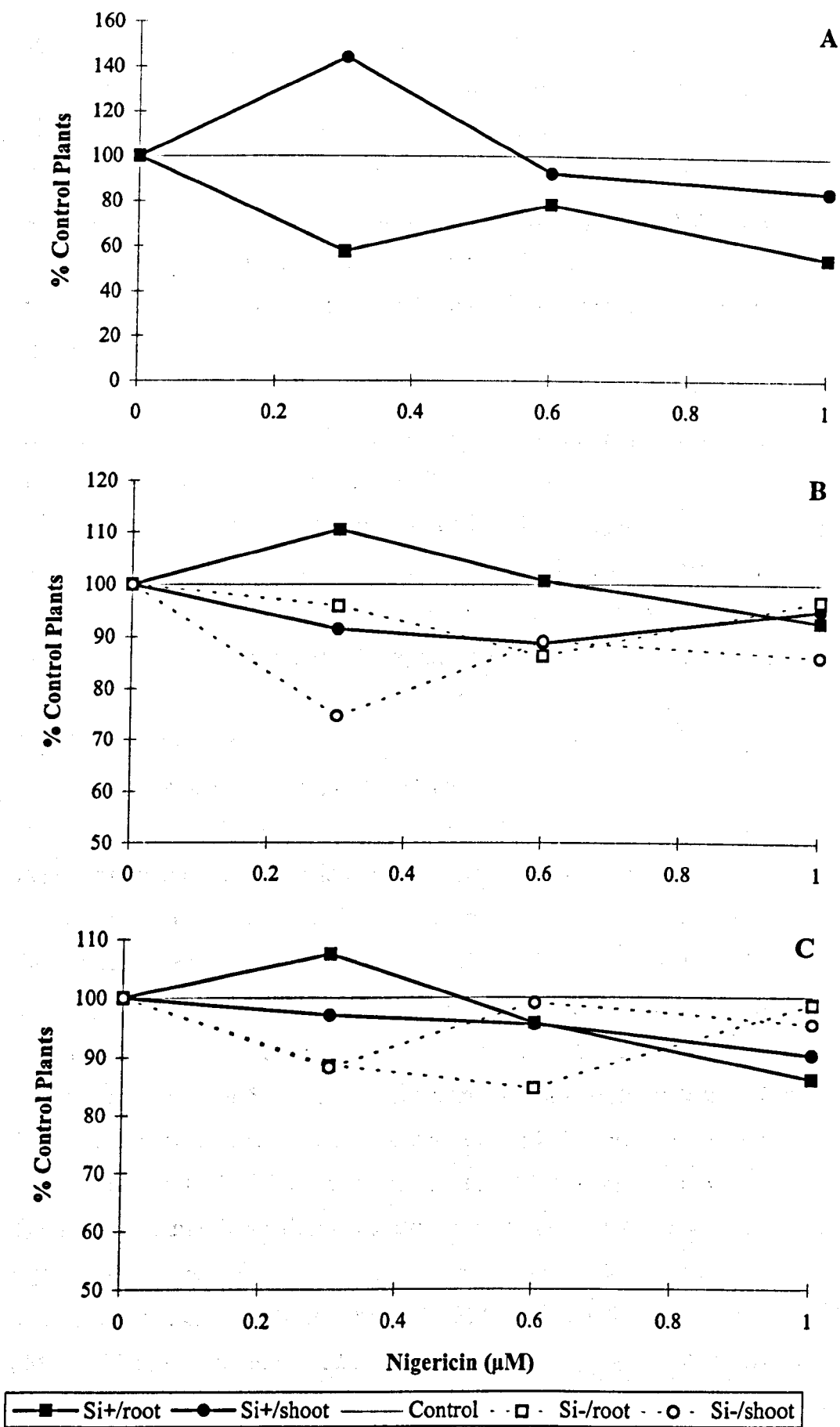
The effect of nigericin on the silica content of wheat plants grown in nutrient solution for 24 hours. The nutrient solution contained 2 mM silica. The control plants were grown in identical nutrient solutions minus the nigericin. Mean average of 4 replicates.

**Figure 5.7b.**

The effect of nigericin on the potassium content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the nigericin. Mean average of 4 replicates.

**Figure 5.7c.**

The effect of nigericin on the phosphate content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the nigericin. Mean average of 4 replicates.



## Potassium.

### Results from Si+ Plants.

Nigericin did not appear to affect the potassium content of the shoots of plants, when subjected to concentrations up to 10  $\mu\text{M}$  (data for concentrations up to 1  $\mu\text{M}$  presented in fig. 5.7b). The lowest silica content occurred when the concentration of nigericin was 0.6  $\mu\text{M}$ , it was reduced to 88.7% of the potassium content of the control plants. 10  $\mu\text{M}$  nigericin did not affect the potassium content greatly, the mean percentage was reduced to 96.7% of the controls.

The roots were slightly more affected by nigericin than the shoots. Nigericin concentration of 0.3  $\mu\text{M}$  produced a <sup>rise</sup> raise in potassium content, <sup>reduced</sup> reduced to 110.50% of control plants (fig. 5.7b). Then the potassium content declined with increasing nigericin concentration until, at 1  $\mu\text{M}$  nigericin, the potassium content was 92.67% of control. When the nigericin concentration was increased to 10  $\mu\text{M}$  the potassium content was increased to 111.12% of control plants (data not presented graphically).

### Results from Si- Plants.

The potassium content of Si- plants displayed a similar trend to the results obtained with the Si+ plants, although the percentage of potassium of the control plants was lower in the Si- plants (fig. 5.7b). All the concentrations of nigericin used in the investigation caused a reduction in the percentage potassium compared to the control plants. This is in slight contrast to the Si+ plants, where in the roots, nigericin concentrations lower than 1  $\mu\text{M}$  somewhat increased the potassium content (see above).

The shoots, of Si- plants, displayed a drop in potassium content (fig. 5.7b) when exposed to nigericin, in the presence of 0.3  $\mu\text{M}$  nigericin there was a reduction to 74.7% of the control plants. Calculations from the  $\mu\text{moles potassium/gDWT}$  data demonstrated that this reduction was significant (d.f.=6,  $p=0.05$ ,  $t=2.552$ ). This was followed by an increase, up to 89.1% of the control plants, when 0.6  $\mu\text{M}$  nigericin was used. Both 1  $\mu\text{M}$  and 10  $\mu\text{M}$  nigericin caused a decline in potassium content of the wheat

plants. Using 1  $\mu\text{M}$  the potassium content of the plants was lowered to 86.0%, with 10  $\mu\text{M}$  (data not presented graphically) it was lowered to 80.1% of the control plants.

The roots displayed less reduction in potassium content when exposed to nigericin in the concentration range 0 - 10  $\mu\text{M}$  than the shoots (data for concentrations up to 1  $\mu\text{M}$  presented in fig. 5.7b). There was slight reduction when the plants were exposed to 0.3  $\mu\text{M}$  nigericin, to 95.89% of the control plants. A greater decrease was observed when the plants were subjected to 0.6  $\mu\text{M}$  nigericin, down to 86.31% of control plants. This was significantly different from the control plants when calculated from  $\mu\text{mole potassium/gDWT}$  data (d.f.= 6,  $p=0.1$ ,  $t=2.187$ ). There followed a slight increase in potassium content of the roots, up to 96.63% of control plants when exposed to 1  $\mu\text{M}$  nigericin. Ten  $\mu\text{M}$  nigericin caused a decline in potassium content to 94.28% of the control plants.

### **Phosphate.**

#### **Results from Si+ Plants.**

Nigericin, in general, appeared to have very little effect on the phosphate content of the wheat plants subjected to 0 - 10  $\mu\text{M}$  concentrations (data for concentrations up to 1  $\mu\text{M}$  presented in fig. 5.7c). The shoots showed an gradual decrease in phosphate content with increasing nigericin concentration. Concentrations of 0.3  $\mu\text{M}$  produced a reduction to 97.1% of the control plants. This decline continued such that 10  $\mu\text{M}$  nigericin caused a decrease of potassium content to 86.8% of control.

The roots displayed an elevated phosphate content when the plants were exposed to 0.3  $\mu\text{M}$  nigericin, up to 107.56% of the control plants (fig. 5.7c). This was followed by a decrease to 95.76% when subjected to 0.6  $\mu\text{M}$  nigericin, and again to 86.07% when using 1  $\mu\text{M}$  nigericin. The potassium content increased slightly following exposure to 10  $\mu\text{M}$ , up to 91.97% of the control plants.

### Results from Si- Plants.

Plants grown without silica in the nutrient solution displayed similar trends of phosphate content to the Si+ plants. The phosphate content of the shoots showed a reduction when exposed to 0.3  $\mu\text{M}$  nigericin, to 88.2% (fig. 5.7c). However, this was followed by an increase to 99.1% of control plants when the plants were subjected to 0.6  $\mu\text{M}$ . The phosphate content then declined when the plant was grown in 1  $\mu\text{M}$  and 10  $\mu\text{M}$  (data for 10  $\mu\text{M}$  not presented graphically) nigericin to 95.4% and 91.6% respectively.

The roots also demonstrated a decreasing phosphate content when subjected to increasing nigericin concentrations, except when the concentration equalled 1  $\mu\text{M}$  (fig. 5.7c). Initially, the phosphate content was reduced to 88.61% of the control plants when exposed to 0.3  $\mu\text{M}$ . This fell to 84.62% when the nigericin concentration was increased to 0.6  $\mu\text{M}$ . When the concentration was further increased to 1  $\mu\text{M}$  there was a raise in phosphate concentration to 98.61% of the control plants. The general downward trend, however, was continued when the nigericin concentration was increased up to 10  $\mu\text{M}$  (data not presented graphically). This produced a reduction in phosphate content to 83.61% of the control plants.

### SUMMARY.

Generally, nigericin at the concentrations up to 10  $\mu\text{M}$  caused a decrease in the silica content of both roots and shoots, more so in the roots. Nigericin had very little effect on the potassium content of the wheat plants, although at 10  $\mu\text{M}$ , Si+ plants appeared to be less affected by the nigericin. Phosphate content tended to be reduced by the presence of nigericin in the nutrient solution. This is equally true for both Si+ and Si- plants.

### 5.3.5e. The Effect of FCCP on the Ion Content of Wheat Plants.

#### Silica.

When wheat plants were exposed to FCCP in the concentration range 0 - 100  $\mu\text{M}$  for 24 hours, there was a decrease in the quantity of silica in the shoots compared to the control plants which had not been



exposed to the FCCP (fig. 5.8a). There was a reduction in silica content to 41.7% in comparison with the control plants when the plants were subjected to 25  $\mu$ M FCCP. This result was significant when calculated from  $\mu$ moles silica/gDWT data (d.f.=6,  $p=0.05$ ,  $t=5.079$ ). There followed a slight increase in silica content, up to 54.9% of control plants when the FCCP concentration was increased to 50  $\mu$ M. This was also a significant difference from the silica content within the control plants (d.f.=6,  $p=0.05$ ,  $t=3.42$ ). When the FCCP concentration was increased to 100  $\mu$ M, there was a further rise in the silica content of the shoots up to 61.4% of the control plants. However, when calculated from  $\mu$ moles silica/gDWT data, this was still significantly different from the control plants (d.f.=6,  $p=0.05$ ,  $t=3.161$ ). Therefore it appeared that, over the concentration range used, FCCP significantly reduced the quantity of silica within the shoots of wheat plants.

Conversely, FCCP increased the quantity of silica within the roots of wheat plants exposed to it for 24 hours (fig. 5.8a). When the plants were subjected to 25  $\mu$ M FCCP, the silica content was over double that of the control plants (203.13%). This increase was significant, when expressed as  $\mu$ moles silica/gDWT (d.f.=6,  $p=0.05$ ,  $t=2.711$ ). As the concentration of the FCCP was increased, the silica content gradually decreased. When exposed to 75  $\mu$ M FCCP the silica content was 113.3% of the control plants and when the FCCP concentration was further increased to 100  $\mu$ M the silica content declined to 107.80%. Neither of these results were significantly different from the silica content of the control plants.

## **Potassium.**

### **Results From Si+ Plants.**

Increasing FCCP concentration generally caused a decrease in potassium content in the shoots of the plants in comparison to the control plants (fig. 5.8b). At 25  $\mu$ M FCCP concentration, the potassium content was reduced to 78.8% of the control plants. This reduction was not significant when calculated from  $\mu$ moles potassium/gDWT data (d.f.=6,  $p=0.10$ ,  $t=1.955$ ). The quantity of potassium was further reduced when the

**Figure 5.8.** The effect of FCCP on the silica, potassium and phosphate content of wheat plants grown in nutrient solution for 24 hours.

**Figure 5.8a.**

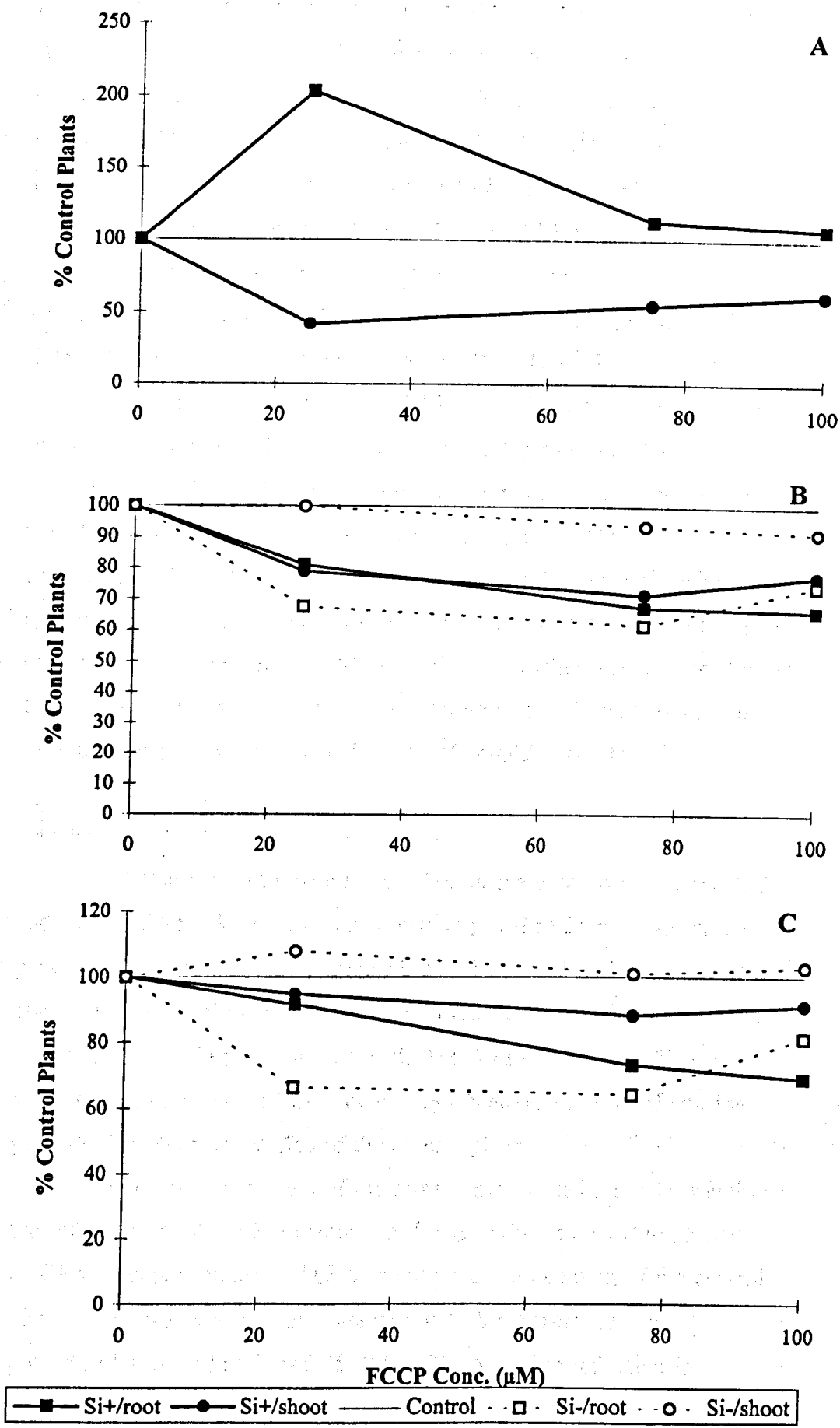
The effect of FCCP on the silica content of wheat plants grown in nutrient solution for 24 hours. The nutrient solution contained 2 mM silica. The control plants were grown in identical nutrient solutions minus the FCCP. Mean average of 4 replicates.

**Figure 5.8b.**

The effect of FCCP on the potassium content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the FCCP. Mean average of 4 replicates.

**Figure 5.8c.**

The effect of FCCP on the phosphate content of wheat plants grown in nutrient solution for 24 hours. The Si + nutrient solution contained 2 mM silica, Si - solution contained no additional silica. The control plants were grown in identical nutrient solutions minus the FCCP. Mean average of 4 replicates.



FCCP concentration was increased to 75  $\mu\text{M}$ , down to 71.4% of control. Again this was a significant decline compared to the control plants (d.f.=6,  $p=0.05$ ,  $t=3.007$ ). There followed a small increase in potassium content when the FCCP concentration was elevated to 100  $\mu\text{M}$ . The plants contained 77.5% of the potassium content of the control plants, although this was still a significant reduction (d.f.=6,  $p=0.05$ ,  $t=2.458$ ).

The quantity of potassium in the roots of the plants followed a similar trend to that of the shoots when the plant was exposed to FCCP (fig. 5.8b). When the plants were subjected to 25  $\mu\text{M}$  FCCP for 24 hours, the potassium content was decreased to 82.72% of the control plants. However this decrease was not significant when calculated from  $\mu\text{moles potassium/gDWT}$  data. The potassium content continued to decline when the FCCP concentration was increased up to 75  $\mu\text{M}$ . The plants then contained 70.53% of the potassium that the control plants contained. A further decrease was observed when the plants were subjected to 100  $\mu\text{M}$  FCCP concentration, down to 67.26% of the potassium in the control plants. This decrease was significant in comparison to control plants (calculated from  $\mu\text{moles potassium/gDWT}$  data, d.f.=6,  $p=0.05$ ,  $t=4.149$ ).

#### Results From Si- Plants.

FCCP appeared to have little effect on the potassium content of the shoots (fig. 5.8b). When the plants were exposed to 25  $\mu\text{M}$  FCCP, the potassium content was barely affected, only being reduced to 99.8% of the control plants. A further increase in the FCCP concentration produced a greater reduction in potassium content, 93.6% of the control. This was again observed when the FCCP concentration equalled 100  $\mu\text{M}$  and the potassium content was 91.7% of the control plants.

The potassium content of the roots demonstrated a similar trend to that of the roots of the Si+ plants (fig. 5.8b). When exposed to 25  $\mu\text{M}$  FCCP the roots contained 67.08% of the potassium content of the control plants. This was a significant decrease, calculated from  $\mu\text{moles potassium/gDWT}$  (d.f.=6,  $p=0.05$ ,  $t=6.102$ ). A further reduction in

potassium content occurred when the FCCP concentration was increased to 75  $\mu$ M, down to 62.21%. This was again significantly different from the control plants when  $\mu$ moles potassium/gDWT was used (d.f.=6,  $p=0.05$ ,  $t=5.856$ ). There followed a slight increase in the quantity of potassium in the roots when the FCCP concentration was elevated to 100  $\mu$ M, up to 74.47% of control. This was not a significant reduction from the control plants (d.f.=6,  $p=0.1$ ,  $t=2.071$ , calculated from  $\mu$ moles potassium/gDWT data).

### **Phosphate.**

#### **Results From Si+ Plants.**

FCCP produced only a negligible affect on the phosphate content of the shoots of plants exposed for 24 hours (fig. 5.8c). FCCP, at a concentration of 25  $\mu$ M, caused a reduction in the quantity of phosphate to 94.8% of that contained within the control plants. On increasing the FCCP concentration to 75  $\mu$ M a further slight decrease was observed, down to 71.4% of control plants. However, when the FCCP concentration was elevated to 100  $\mu$ M there was a slight rise in phosphate content of the shoot, up to 77.5% of the control plants. None of the above changes in the phosphate content were significantly different from the control plants.

The reduction in phosphate content of the roots of plants exposed to FCCP concentrations in the range 0 - 100  $\mu$ M was greater than that demonstrated by the shoots of the plants (fig. 5.8c). However, the roots only displayed a slight reduction when subjected to 25  $\mu$ M FCCP, 92.97% of the control plants. This was similar to the response shown by the shoots. When the FCCP concentration was increased to 75  $\mu$ M, the decline in phosphate content equalled 74.53% of control plants. This decline was slightly significant in comparison to the control plants when calculated from  $\mu$ moles phosphate/gDWT results (d.f.=6,  $p=0.01$ ,  $t=1.975$ ). A further drop in the quantity of phosphate in the roots of the plants occurred when the FCCP concentration was elevated to 100  $\mu$ M, to 69.16% of the control plants. This was a significant decrease in comparison to the control plants

(d.f.=6,  $p=0.05$ ,  $t=3.613$ ).

#### Results From Si- Plants.

Again the shoots showed very little response to FCCP in the nutrient solution (fig. 5.8c). The quantity of phosphate increased to 107.6% of the control plants, although this was not a significant increase. When the plants were subjected to 75  $\mu\text{M}$  and 100  $\mu\text{M}$  FCCP there was very little alteration in the phosphate content, 101.0% and 103.0% of the control plants respectively.

The roots displayed a greater response to FCCP than the shoots (fig. 5.8c). 25  $\mu\text{M}$  FCCP caused a reduction of phosphate content to 66.81% of the control plants. This was a significant reduction calculated from the  $\mu\text{moles phosphate/gDWT}$  data (d.f.=6,  $p=0.05$ ,  $t=3.362$ ). There was only a slight further decrease in the quantity of phosphate in the roots when exposed to 75  $\mu\text{M}$ , 65.46% of the control plants. When the FCCP concentration was elevated to 100  $\mu\text{M}$ , there was a rise in phosphate content, up to 81.26% of the control plants. This was not significantly different from the control plants.

#### SUMMARY.

At low concentrations, FCCP appeared to reduce the silica content in the shoots, but increase it within the roots. This effect was lost as the FCCP concentration was increased. FCCP reduced the potassium content in Si- and Si+ plants, although Si- shoots were less affected. High concentrations of FCCP caused a decline in the phosphate content of the plants, although again, Si- shoots were the least affected.

#### 5.3.5f. Excised Roots.

The results of the investigation using excised roots are presented in table 5.3. The roots were subjected to the experimental solution for 3 hours. There was a significantly greater quantity of silica present in the excised roots which had been incubated in 2 mM silica nutrient solution

**Table 5.3.** A comparison of the silica within excised roots exposed to 2 mM silica in a full nutrient solution for 3 hours with and without the presence of erythrosin B.

Silica Concentration (mM)	Erythrosin B Conc. (μM)	μmoles Silica/ g FWT ± std.
0	0	0.737 ± 0.483 (n = 10)
0	1	0.595 ± 0.460 (n = 6)
2	0	1.081 ± 0.421 (n = 10)
2	1	0.769 ± 0.237 (n = 6)

than in the Si- roots (d.f.=18,  $p=0.1$ ,  $t=1.607$ ). When EB was included in the experimental solution there was a significant reduction of the silica content within the excised roots (d.f.=14,  $p=0.1$ ,  $t=1.560$ ). EB did not have any significant effect on the silica content of Si- roots (d.f.=14,  $p=0.1$ ,  $t=0.75$ ).

#### 5.3.5g. Calculated Results From Section 5.3.5.

Using dry weight data acquired after harvesting the plants of the inhibitor investigation, it was possible to calculate the mean average number of  $\mu$ moles of silica present in one plant. Thus, the effect an inhibitor had on the distribution of silica within the plant could be calculated.

One concentration per inhibitor was used for the calculation. The concentration to be used was the concentration of inhibitor where there was a significant change in the silica content of the root, or if the inhibitor did not affect the root content, then the concentration which caused a significant change in the shoot content. Ideally, a concentration which significantly altered the silica content of both shoot and root should be used.

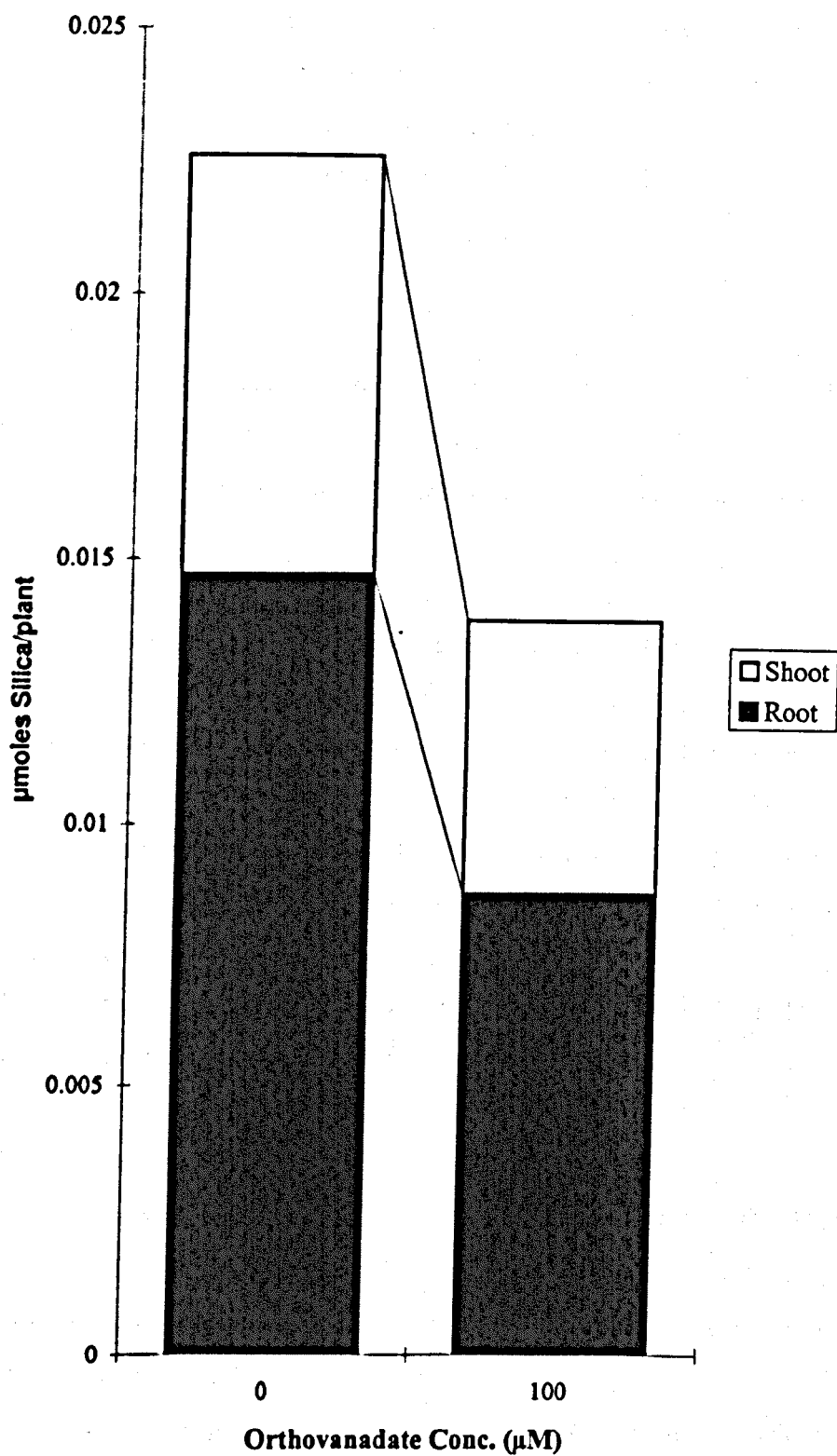
It should be noted that reference to total silica within the plant excludes silica within the caryopsis. Previous investigations (see section 5.3.4) have demonstrated that this silica is present in Si- plants and is therefore a contaminant.

#### **Vanadate.**

The sodium orthovanadate concentration which produced a significant decrease in root silica was  $100 \mu\text{M}$ , the highest concentration used in this investigation.

The number of  $\mu$ moles of silica per plant organ are given in figure 5.9. It can be seen that the vanadate produced a reduction in total silica present within the plant which had been exposed to  $2 \text{ mM}$  silica nutrient solution. There is a 39 % reduction in silica uptake. The partitioning of the silica is only slightly changed. When the plant is not subjected to vanadate 35 % of the total silica is present in the shoot and 65 % in the





**Figure 5.9.** The effect of 100  $\mu\text{M}$  orthovanadate on the silica partitioning between the roots and shoots of a wheat plant exposed to 2 mM silica for 24 hours.

root. However, when 100  $\mu\text{M}$  vanadate was present in the nutrient solution, this partitioning was only slightly altered. Thirty-seven % of the total silica was present in the shoots and 63 % in the roots. Therefore it appears that at this concentration of orthovanadate the major effect of the inhibitor is to reduce the uptake of silica in to the plant.

### **Erythrosin B.**

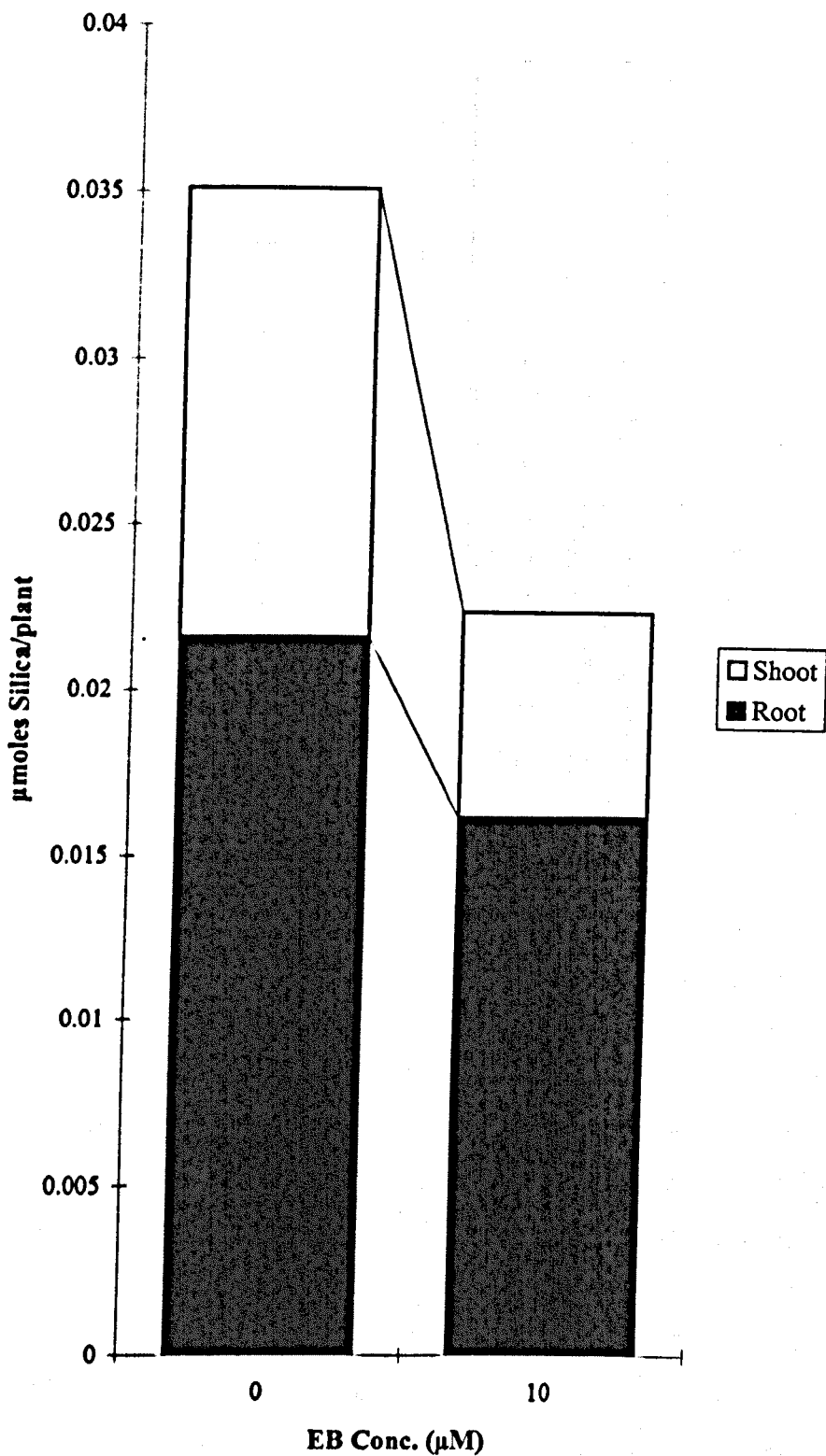
The concentration used for the calculations was 10  $\mu\text{M}$ . This was the highest concentration used in the present investigation, and at this concentration there was a significant reduction in the silica content of the shoots.

The number of  $\mu\text{moles}$  of silica per plant organ are given in figure 5.10. It is quite clear that the EB caused a reduction in the total silica content of the whole plant. There is a 36 % reduction compared to control plants. The partitioning of the silica within the plant also changes slightly in the presence of the inhibitor. The control plants contain 38 % of the total silica within the shoots and 62 % in the roots. When subjected to the inhibitor the percentage within the shoots was reduced to 28 % of the total and consequently the root were increased to 72 %. Therefore it appears that EB had two effects on the silica content of the wheat plants, reduction of uptake and reduction of silica within the shoot.

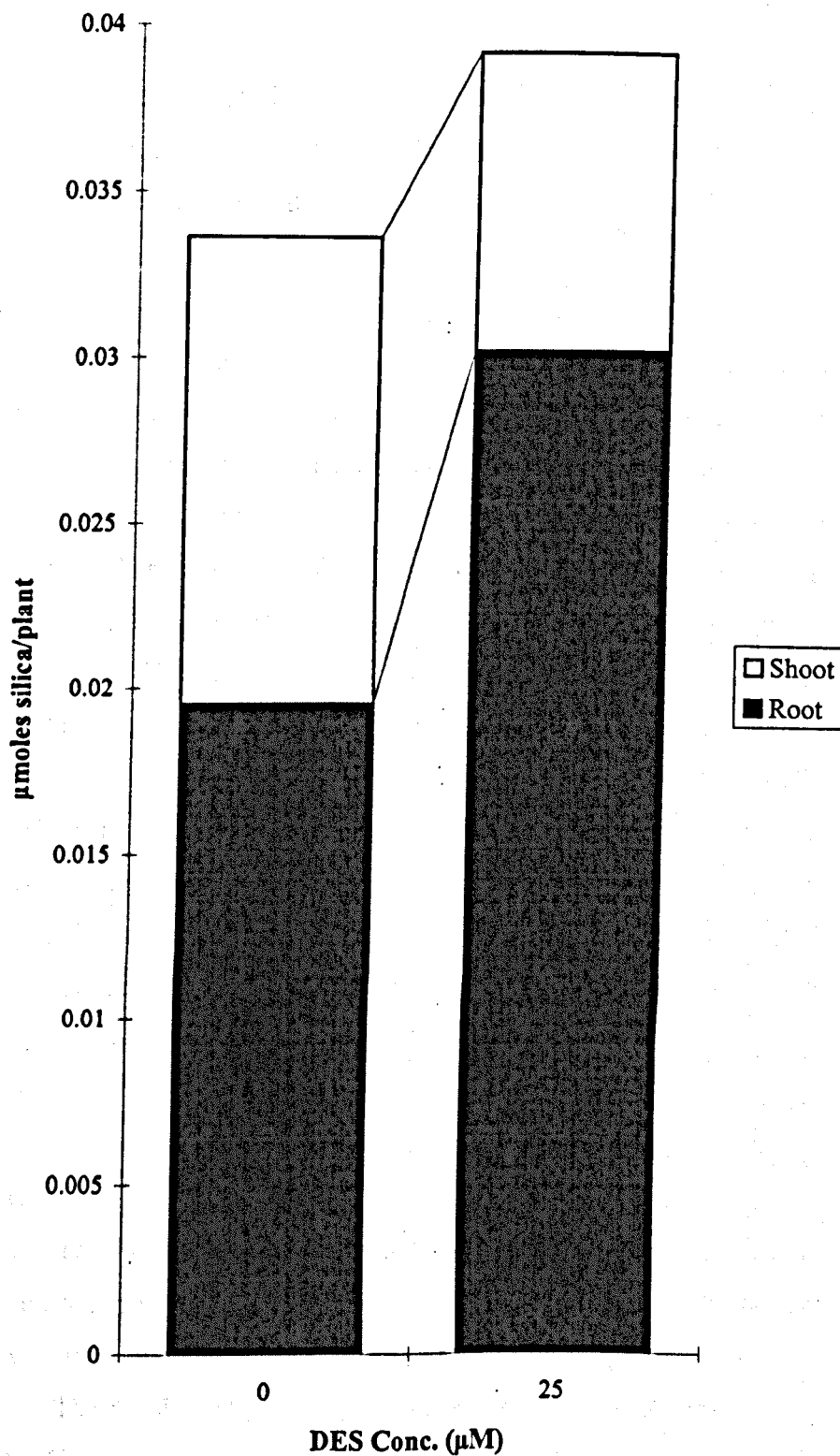
### **DES.**

The concentration used for the calculations was 25  $\mu\text{M}$ . This concentration of inhibitor significantly altered the silica content of both roots and shoots. The number of  $\mu\text{moles}$  of silica per plant organ are presented in figure 5.11.

The total quantity of silica within the whole plant was slightly increased when the plant was exposed to 25  $\mu\text{M}$ , by 17 %, in comparison to the control plants. The greatest effect of the inhibitor, however, appeared to be the alteration of the silica partitioning within the plant. In the control plants, 42 % of the total silica was present within the shoots and, thus, 58%



**Figure 5.10.** The effect of 10  $\mu\text{M}$  erythrosin B on the silica partitioning between the roots and shoots of a wheat plant exposed to 2 mM silica for 24 hours.



**Figure 5.11.** The effect of 25  $\mu\text{M}$  DES on the silica partitioning between the roots and shoots of a wheat plant exposed to 2 mM silica for 24 hours.

within the root. When the plants were grown in the presence of 25  $\mu\text{M}$  DES the percentage of silica within the shoot was reduced to 23 % of the total silica (consequently, the root increased to 77 % of the total silica). It seems that the major effect of DES on the silica content of wheat plants was to reduce the percentage of total silica within the shoots.

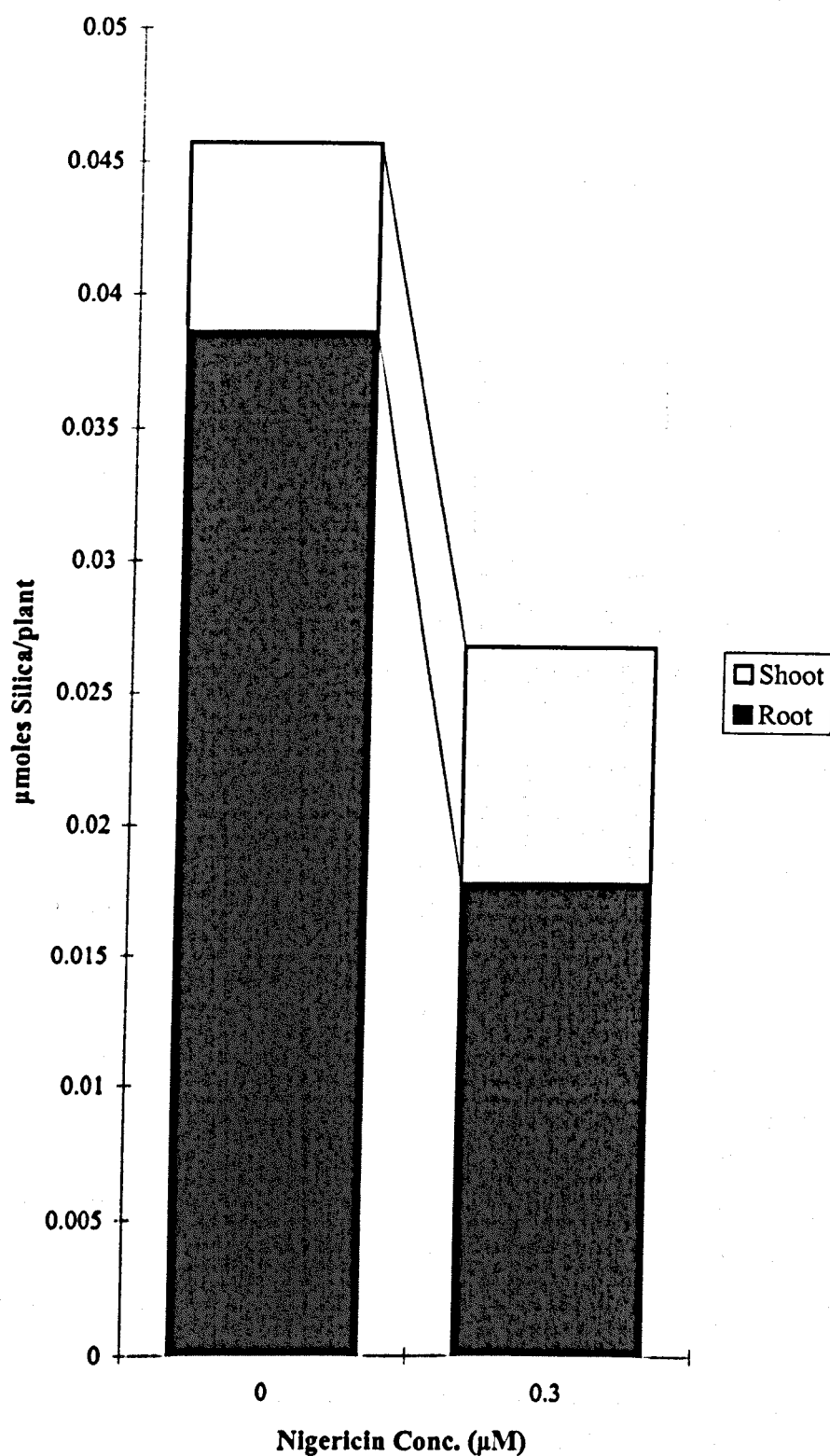
### **Nigericin.**

The concentration of nigericin which produced a significant decrease in root silica content was 0.3  $\mu\text{M}$ . The number of  $\mu\text{moles}$  of silica per plant organ is presented in figure 5.12. The control plants appear to partition a greater percentage of total silica to the roots compared to control plants from the other inhibitor studies. The reason for this is unclear. Nigericin produced a decrease in the total silica content of the plants. This was a reduction of 42 % in comparison with plants which had not been exposed to the inhibitor. There was also a change in the silica partitioning within the plant exposed to 0.3  $\mu\text{M}$  nigericin. Initially, when not subjected to inhibitor, 16 % of the total silica was present in the shoots and 84 % in the roots. However when the plants were exposed to 0.3  $\mu\text{M}$  nigericin the partitioning changed, 33 % was in the shoot and 67 % of the total silica was in the roots (this appears to be a more typical partitioning of silica). The greatest effect of nigericin on the plant silica appears to be the reduction in uptake.

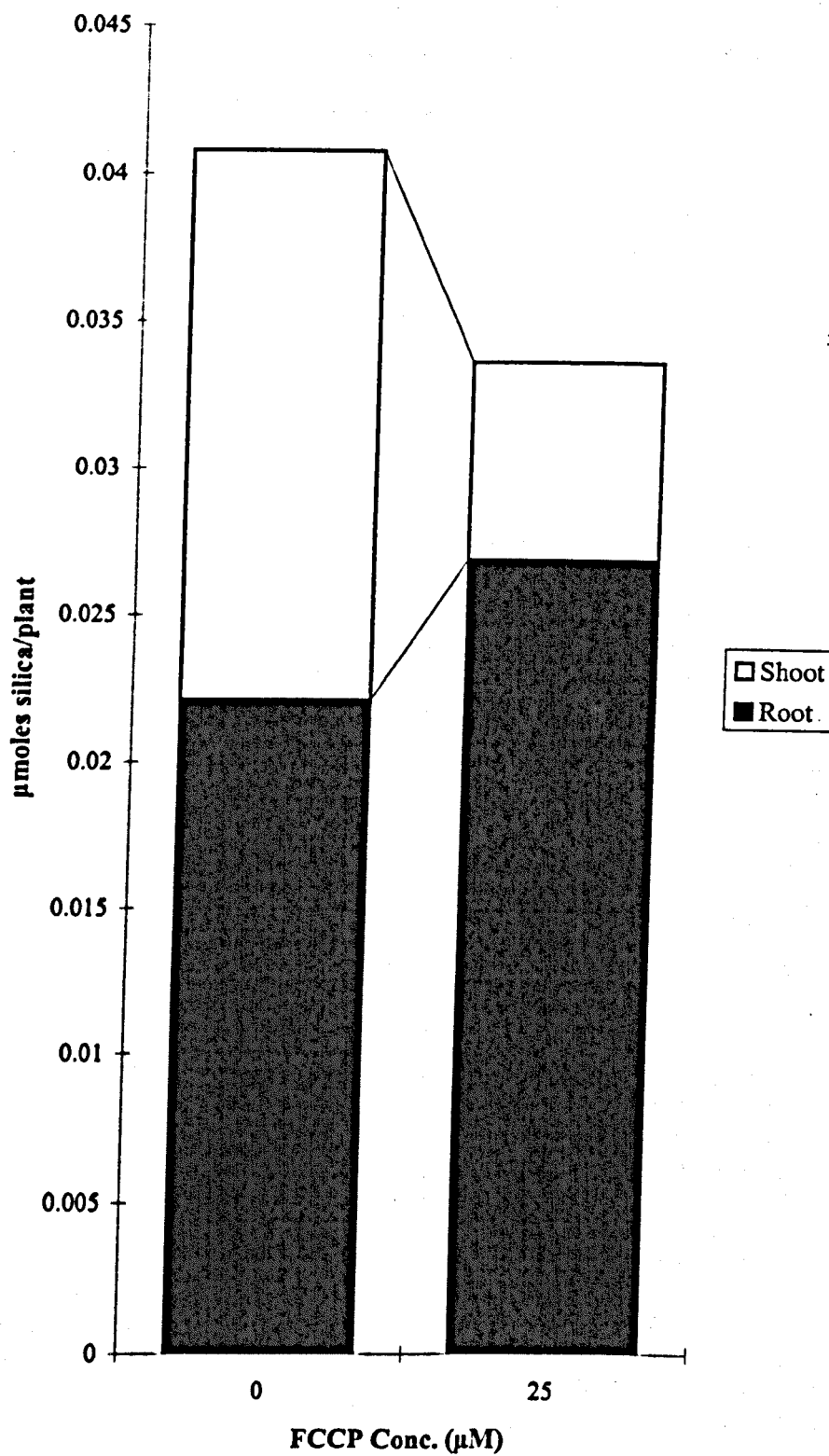
### **FCCP.**

The FCCP concentration used for the calculation was 25  $\mu\text{M}$ . This concentration produced a significant change in both the roots and shoots of the wheat plants.

The number of  $\mu\text{moles}$  of silica per plant organ is given in figure 5.13. There is a slight reduction in the total content of plants exposed to 25  $\mu\text{M}$  FCCP, an 18 % reduction. However, there is a greater change in silica partitioning when subjected to 25  $\mu\text{M}$  FCCP. In the control plants 46 % of the total silica was within the shoot and 54 % in the root. This distribution



**Figure 5.12.** The effect of 0.3  $\mu\text{M}$  nigericin on the silica partitioning between the roots and shoots of a wheat plant exposed to 2 mM silica for 24 hours.



**Figure 5.13.** The effect of 25  $\mu\text{M}$  FCCP on the silica partitioning between the roots and shoots of a wheat plant exposed to 2 mM silica for 24 hours.

changed when the inhibitor was present. Twenty percent of the total silica was within the shoot and 80 % was within the root. Therefore the major effect of the FCCP appeared to be a reduction in the quantity of silica within the shoot.



## 5.4. Discussion.

This was a preliminary study on the nature of silica uptake in young wheat seedlings. A discussion of the separate investigations will be followed by a proposed mechanism for silica uptake based upon the present results.

### 5.4.1. The Effect of Silica Concentration

Results from the present investigation indicated that over the concentration range 0 - 5 mM Si, the roots and shoots of the wheat seedlings demonstrated an increasing accumulation of silica with increasing silica supply (fig. 5.1). Van der Vorm (1980) reported on the effect of three Si concentrations (0.01, 0.5 and 2.7 mM) on various plant species, including wheat. The results showed that at higher Si concentrations the plants contained greater amounts of Si although the increase was not linear. Similar results presented by Jarvis (1987) demonstrated that a doubling of the silica supply from 0.71 to 1.42 mM did not double the silica content of wheat plants. These published reports compare well with the results presented in this chapter. The present results displayed a levelling off of the rate of silica accumulation when Si concentration exceeded 2 mM. There are several possible reasons. If Si enters the symplasm of the root by a carrier system (which is likely, considering its low solubility in membranes [Raven, 1983]), either through an ion channel or a porter, this may become saturated at high Si levels. Alternatively, at high concentrations the solubility of silica may decrease and thus less Si would be available for uptake (assuming only monosilicic acid is available to plants). The theoretical limit for silicic acid solubility is about 100 ppm (3.5 mM, Iler, 1955). Analysis of the nutrient solution to determine soluble silica concentrations might have clarified this suggestion.

Considering the results of the present investigation and those of Van der Vorm (1980) and Jarvis (1987), a 2 mM Si concentration was thought to be a suitable concentration for uptake studies. In addition, when supplied at this concentration, Si was taken up by wheat plants in quantities which

could be reliably detected by the molybdate reaction.

#### 5.4.2. 24 Hour Time Course of Silica Uptake.

A plant should be exposed to an inhibitor for a short as time as possible, therefore a time course for silica uptake was conducted. The present results demonstrated that silica accumulation into the root was linear over the range of 3 - 24 hours exposure to 2 mM Si (fig. 5.2), on average, 2.74  $\mu\text{molesSi/gDWT/hour}$  was accumulated by roots. The shoots however, displayed a less rapid accumulation that appeared to level off after 12 hours. A possible reason for this may be that this period included a 6 hour night cycle at 15°C (see section 3.2.1). This may cause a reduction in transpiration and hence transport to the shoot and, possibly, a small reduction in root uptake. Several possibilities can be put forward concerning this reduction in transport. Silica transport to the shoot is thought to occur in the xylem. During the night cycle the transpiration rate of the plants is greatly reduced and a decline in xylem transport may be expected. Alternatively, if xylem loading is an active process lowering of the temperature might be responsible for the observed reduction in Si transport. It is unclear why silica transport to the shoot is more affected than uptake. If silica uptake is active then the decrease in temperature would be expected to slow down this process as well as transport. The present results are supported by the work of Barber & Shone (1966). They demonstrated that a temperature change from 25°C to 5 °C applied to the roots virtually halved the Si content of the shoots of barley over 24 and 48 hours. In the present investigation, during the night cycle, in addition to the decline in temperature the plants were exposed to 6 hours darkness. Silica transport in rice (reviewed by Okuda & Takahashi, 1965), did not depend on light as much as phosphorus and potassium. There are many physiological changes occurring in the plant during the night cycle, and further work would be needed to determine their precise effect on silica uptake and transport.

There are a few studies concerning Si uptake over relatively short

time periods. Rothbur & Scott (1957) used  $^{31}\text{Si}$  to follow Si uptake in wheat plants over 12 hours. After 4 hours "leaves" contained 0.52  $\mu\text{molesSi/g}$  of dried plant and roots contained 1.08  $\mu\text{molesSi/g}$  of dried plant. They used 0.21 mM  $^{31}\text{Si}$ . In comparison, the results presented currently demonstrate after 3 hours exposure to 2 mM Si, shoots contained 1.85  $\mu\text{molesSi/gDWT}$  and the roots contained 13.76  $\mu\text{molesSi/gDWT}$ . The difference between these results and those of Rothbur & Scott (1957) may be explained by the difference in silica concentrations in the nutrient solutions. The current results were obtained using 10 times the silica concentration and the silica content of the roots observed were of the order of 10 times greater than those of Rothbur & Scott. Other investigators have studied the effect of time on the silica content of plants. Lowland rice after 24 hours exposure to silica contained 20  $\mu\text{molesSi/plant}$  (recalculated from 1.2 mgSiO<sub>2</sub>/plant, fresh weight of plant was about 12 g. Reviewed by Okuda & Takahashi, 1965). Barber & Shone (1966) exposed barley to 1.1 mM Si for 24 hours. Under low humidity, roots contained 30.3  $\mu\text{molesSi/gDWT}$  and shoots contained 64.2  $\mu\text{molesSi/gDWT}$ . The results presented in the current investigation conflict with these results and after 24 hour exposure to 2 mM the silica content of the wheat plant was 53.88 and 6.23  $\mu\text{molesSi/gDWT}$  for roots and shoots respectively. The conflict in the results may be due to differences among the species investigated or humidity, although silica content of barley is unaffected by humidity.

#### 5.4.3. The Effect pH on Silica Uptake.

A pH optimum for silica uptake appeared to be elusive in the literature. Most references for pH concerned the reaction of Si with soil particles at various pH values (see Jones & Handreck, 1965). However, the use of water culture allowed an investigation into pH effects without the complications of soil interactions. The present results suggest that Si uptake has a broad pH optimum, between 5.2 - 7.2 (fig. 5.3), and that outside this range silica content of the roots fell rapidly. The shoot silica levels did not show variation with pH. This is probably because transport to the shoot is

unlikely to depend on the pH of the nutrient solution. The results suggest the possibility that transport to the shoot is independent of the silica status of the root. At pH 3.2 and 9.2 root silica content is considerably reduced but the shoot silica content appeared unaffected.

It is not unexpected that Si uptake is pH-dependent as many physiological processes have pH optimums. It must be stressed, however, that the experiments were conducted on whole plants and that the optimum observed is the result of many interacting systems. This may explain the apparent broad optimum.

In the literature, solutions set at various pH have been used. For example: Van der Vorm (1980) used pH  $5.2 \pm 0.1$  for all the species investigated; Si solutions used by Rothbur & Scott (1957) were pH 5.5; Ma & Takahashi (1993) set pH at 5.5 using KOH; and Barber & Shone (1966) only stated that their solutions were below pH 7. Mayland, Wright & Sojka (1991) measured soil pH as 8.0 (1:1 soil:water) and the present results suggest that this is beyond the pH optimum for silica uptake for wheat, without considering the soil-silica interactions.

The pH of the solution affected Si content of the roots of the wheat seedlings, however, this may not be due to a direct effect on silica uptake. No other elements or parameters were measured in the plants after the pH treatments, and thus a general disturbance in plant physiology can not be ruled out. The investigation used full nutrient solutions, and therefore interactions between silica and other nutrients in solution may change as the pH is altered and affect the availability of silica for uptake.

Silica solubility is stable over the pH range 3 - 8, increasing at pH 10 (Alexander, Heston & Iler, 1954). It would seem unlikely that a change in silica solubility was a possible reason for the pH optimum observed. The effects of other ions on silica solubility and speciation over the pH range is unknown, however.

A further consideration to be taken into account is that the pH of the solutions was set using NaOH and HCl. Typically, when the metasilicate solution was added to the nutrient solution, the pH was very high, in the

range of pH 10 - 12. Plants growing in low pH solutions were, therefore, exposed to higher concentrations of chloride ions than plants growing at higher pH solutions. The effect of the chloride ion concentration on silica uptake is a factor which may have influenced the results. The pH optimum measured only applies when 2 mM Si is supplied. Other pH optima were not determined using different Si concentrations.

The investigations presented above were an initial study to determine optimum conditions for silica accumulation in wheat seedlings. These conditions would then be used to determine the effects of inhibitors on uptake and transport of Si in wheat.

#### 5.4.4. Transpiration Experiment.

It was determined that the cultivar of wheat used in the current investigation, Beaver, did actively accumulate Si by calculating the transpiration stream concentration factor (TSCF) for plants grown for 24 hours in 2 mM Si at pH 5.2 in full nutrient solution (tab. 5.2). TSCF equalled 1.3, and thus it was concluded that wheat cultivar Beaver accumulated Si by means other than mass flow alone, under the specified conditions. Van der Vorm (1980) reported that for wheat, "actual Si uptake : mass flow Si" ratios calculated for 0.01 mM, 0.5 mM and 2.7 mM Si were 5.5, 2.1 and 0.9 respectively. These values compares favourably with the results reported currently. TSCF is above unity (indicating active uptake) although, a large proportion of Si may passively enter the plant, and a small quantity by "alternative" means. This is important when considering the results from the inhibitor studies. If a large amount of Si enters the plant passively, the inhibitors used would not be expected to produce a complete inhibition of silica accumulation. A lower concentration of silica should, ideally, have been used as then a larger portion of Si may be entering actively. The lower overall levels of Si within the plant were, however, difficult to measure consistently with the molybdate blue method and  $^{31}\text{Si}$  was difficult to obtain and too expensive to be used.

#### 5.4.5. The Effect of Inhibitors on Ion Content.

Based upon the results discussed above, the time period of 24 hours was considered to be the most suitable time period for the inhibitor studies. Silica content could be reliably determined after this period. Previously reported times for exposure to inhibitors vary. Corn seedlings were subjected to various inhibitors for only 3 - 4 hours (Lüttge & Laties, 1967). Germinating radish seedlings were exposed to orthovanadate for 20 hours (Cocucci, Ballarin-Denti & Marré, 1980). Indeed, Barber & Shone (1966) subjected barley seedlings to 2,4 dinitrophenol or sodium azide for 24 hours. Rice plants were grown for 24 and 48 hours in various metabolic inhibitors (reviewed by Okuda & Takahashi, 1965).

The standard conditions for the inhibitor studies were a full nutrient solution containing 2 mM sodium metasilicate set at pH 5.2 containing an inhibitor for 24 hours. The five inhibitors used were orthovanadate, erythrosin B (EB), diethylstilbestrol (DES), nigericin and FCCP. The potassium and phosphate content of the plants was recorded in addition to silica content, this was to allow the effect of the inhibitors on known uptake systems to be monitored. The results of each inhibitor will be discussed first followed by an overview of the results.

##### 5.4.5a. Orthovanadate.

This has been proven by various investigations to be an ATPase inhibitor. In the present investigation, 100  $\mu$ M orthovanadate caused a reduction in potassium content of both roots and shoots of both Si<sup>-</sup> and Si<sup>+</sup> plants (fig. 5.4b). This suggests that orthovanadate was inhibiting the H<sup>+</sup>-ATPase and thus decreasing the potassium uptake by the plants. The results compare well with published reports where orthovanadate concentrations of 100  $\mu$ M (Cocucci *et al.*, 1980), 200  $\mu$ M (Colombo *et al.*, 1981) and up to 500  $\mu$ M (Gallagher & Leonard, 1982) have inhibited potassium and proton transport in plant material. The concentration of orthovanadate used in the present investigation may have been too low considering the above reports. Over the lower concentration ranges the potassium content of Si<sup>+</sup> plants

appeared to be slightly less affected by orthovanadate than in Si- plants. At 100  $\mu\text{M}$  orthovanadate there was little effect on phosphate content in Si- plants. Si+ plants, however, displayed a slight reduction (fig. 5.4c). A reduction in phosphate content was expected as orthovanadate reduces the proton gradient required for phosphate uptake. One hundred  $\mu\text{M}$  orthovanadate produced 34.8% reduction in shoot Si and a 41.0% reduction in root Si (overall a 39% reduction in total plant silica [fig. 5.4a]). This may suggest that inhibition of  $\text{H}^+$ -ATPases caused, directly or indirectly, a reduction in silica content of wheat seedlings.

#### 5.4.5b. Erythrosin B.

This is also an ATPase inhibitor. EB appeared to cause an increase in root Si content and a decrease in shoot Si content at a concentration of 1  $\mu\text{M}$  (fig. 5.5a). This possibly suggests an inhibition of transport, but not uptake. When EB concentration was increased to 10  $\mu\text{M}$ , a reduction in Si content in both root and shoots was observed, similar to orthovanadate at 100  $\mu\text{M}$ , suggesting that both transport and uptake were inhibited. EB reduces the silica content of both roots and shoots when supplied at 10  $\mu\text{M}$ , however, there does not appear to be a reduction in the potassium content (fig. 5.5b), which would have been expected as EB inhibits  $\text{H}^+$ -ATPase. In fact, there appeared to be an increase in potassium content in the roots of the plants in both Si+ and Si- plants. This is similar to the results for phosphate content of the plants (fig. 5.5c). There did not appear to be any clear differences between Si+ and Si- plants. As EB inhibits the vanadate sensitive  $\text{H}^+$ -ATPase, these results would be expected to be similar to those obtained for the orthovanadate. It is possible that the concentration of EB used was not sufficient to inhibit the proton ATPase and thus affect potassium transport, but high enough to inhibit silica uptake. In *Ricinus communis* cotyledons sucrose uptake was inhibited 59 % by  $50 \times 10^{-3} \text{mol dm}^{-3}$  (Ball, Williams & Hall, 1987). Cocucci (1986) demonstrated that 100  $\mu\text{M}$  EB produced an 85% inhibition of vanadate-ATPase in microsomal preparations from radish seedlings (10  $\mu\text{M}$  produced a 50%

inhibition), however,  $\text{Ca}^{2+}$ -ATPase was inhibited 50 % by 0.2  $\mu\text{M}$  EB in microsomal preparations from radish (Rasi-Caldogno, Pugliarello & De Michelis, 1987). Also cultivar differences in sensitivity to EB in sorghum root tips were noted by Wilkinson & Duncan (1993), two cultivars required 0.01  $\mu\text{M}$  EB to produce a 33% inhibition of calcium uptake and two other cultivars required 1  $\mu\text{M}$  EB to display the same degree of inhibition. The concentrations of EB required to inhibit  $\text{Ca}^{2+}$ -ATPases are much lower than those required for the inhibition of the PM  $\text{H}^{+}$ -ATPases (De Michelis, Carnelli & Rasi-Caldogno, 1993). However, the affect on silica content in this chapter may not be as a direct response to EB or alternatively, uptake and transport of silica may be more sensitive to EB than proton-ATPases. At low concentrations of EB there appeared to be inhibition of transport to the shoot compared to inhibition of silica uptake, which was also evident at 10  $\mu\text{M}$ . Excised root data from the present investigation demonstrated that 1  $\mu\text{M}$  EB significantly reduced silica uptake (table 5.3). However, the effects on potassium levels were not determined. The silica uptake results from excised root studies tended to support those obtained using orthovanadate for whole plants. ATPase inhibitors appear to inhibit silica uptake and transport.

#### 5.4.5c. Diethylstilbestrol.

This ATPase inhibitor has been used on excised roots extensively by Balke & Hodges (1977, 1979a, b & c). The results from the present study indicate that DES inhibited both potassium and phosphate uptake in  $\text{Si}^{+}$  and  $\text{Si}^{-}$  wheat plants over the whole concentration range, 25  $\mu\text{M}$  - 100  $\mu\text{M}$  (fig. 5.6b and 5.6c). These results compare favourably to the published results for DES inhibiting ATPases. Balke & Hodges (1977) used this concentration range on oat roots. They reported 100  $\mu\text{M}$  DES inhibited potassium absorption to 25.4 % of the control, chloride absorption was reduced to 16.2% of the control and ATPase activity at the PM declined to 49.3% of the control. Inhibition of potassium uptake has been reported using 10  $\mu\text{M}$  DES, and phosphate uptake using 35  $\mu\text{M}$  (Lin, 1979) and



proton extrusion using 50  $\mu\text{M}$  (Colombo *et al.*, 1981) in maize roots. The present results demonstrate that the effect on silica content is, however, different from that on potassium. At low DES concentration, the silica transported to the shoot appeared to be stimulated, although the silica uptake into the root appeared unaffected (fig. 5.6a). When the concentration of DES was increased to 100  $\mu\text{M}$  there was a decrease in silica content of both root and shoots. This may suggest Si uptake is less sensitive to DES than potassium and phosphate uptake. At this concentration (100  $\mu\text{M}$ ), the present results agree with those obtained using orthovanadate and EB, in that ATPase inhibitors appear to inhibit uptake and transport of silica in wheat roots.

#### 5.4.5d. Nigericin.

No references could be found relating to the use of this  $\text{H}^+/\text{K}^+$  exchanger in whole tissues from higher plants. In the present investigation, it was considered that the potassium content of the plants would be reduced if nigericin exchanges  $\text{H}^+/\text{K}^+$ , due to the decline in the proton gradient and the leakage of potassium ions. These results were not observed. Nigericin appeared not to affect either potassium or phosphate content of the wheat plants in either  $\text{Si}^+$  or  $\text{Si}^-$  plants (fig. 5.7b and 5.7c), but silica content was affected (fig. 5.7a). Low concentrations of nigericin, 0.3  $\mu\text{M}$ , appeared to stimulate transport of silica to the shoot without a corresponding increase in silica uptake to the root. High concentrations (1  $\mu\text{M}$ ) caused a general reduction in silica content of the wheat seedling. This is consistent with previous results from the ATPase inhibitor studies, and suggests that a  $\text{H}^+/\text{K}^+$  gradient, generated by  $\text{H}^+$ -ATPase, is required for Si uptake and transport. The concentrations of nigericin used in the present experiments were very low compared to the levels reported in microsomal vesicle studies, and both Sze (1980) and Churchill, Holaway & Sze (1983) used 5  $\mu\text{M}$  nigericin. Nigericin did not affect the initial uptake of silica in the marine diatom *Nitzschia alba* and it was concluded that neither a potassium or proton gradient was required for silica uptake (Bhattacharyya & Volcani,

1980). They concluded that silica was symported with sodium, driven by a sodium gradient maintained by an ATPase pump, which does not appear to be the case in wheat plants.

#### 5.4.5e. FCCP.

This protonophore inhibited the potassium content in the wheat plants over the whole concentration range 25 -100  $\mu$ M (fig. 5.8b). Transport of potassium in Si- plants appeared to be less affected than in Si+ plants and phosphate appeared to be inhibited to a lesser extent (fig. 5.8c). Transport in Si- plants appeared to be less affected, but uptake was more affected than Si+ plants. These current results compare favourably with previous reports. FCCP, at 1  $\mu$ M concentration has been shown to inhibit potassium uptake and transport (Lüttge & Laties, 1967; Lin, 1979) and 1.5  $\mu$ M inhibited phosphate uptake (Lin, 1979). The silica data suggests that, especially at low FCCP concentrations, transport to the shoot is reduced more than uptake of silica uptake at the roots. It is possible that xylem loading of the silica is more sensitive to FCCP than silica uptake, although both processes appear to rely on a proton gradient.

#### In Summary.

The present investigation has shown that inhibitors of ATPases, orthovanadate, DES and EB, at high concentrations reduce the silica content of wheat plants and this is supported by results from excised roots. Typically uptake and transport were reduced, however, at low concentrations of EB there was a greater reduction of transport than uptake. Nigericin and FCCP also reduced the silica content of the wheat plants, although, nigericin appeared to affect uptake greatly whereas FCCP affected transport more.

It is possible that the presence of inhibitors in the nutrient solution may affect the transpiration rate of the plants, although it was not investigated. However, the transpiration experiment (5.3.4) suggested that silica uptake was in excess of that which could be accounted for by mass

flow and therefore suggests silica uptake is independent of transpiration.

A further possibility is that the inhibitors could be affecting the uptake of other ions within the nutrient solution and this in turn may affect silica transport. This requires further detailed analysis to determine if any interrelationships exist between silica and plant nutrients.

There is a general agreement with the review of solute uptake presented in the introduction (5.1), in that uptake and transport (xylem loading) of silica appear to require functional ATPases and proton gradients. Generally silica uptake appeared to be comparable with potassium and phosphate, both of which are actively taken up as charged ions.

5.4.6. A Proposed Model. A proposed model for Si uptake and transport can be put forward based on the present results. The data is very limited and the problems with the investigation will be discussed after the proposed model. The model has to address the following points. Hodson & Sangster (1989c) demonstrated, using X-ray microanalysis, that soluble Si was present in the stele in the central metaxylem lumen, endodermal protoplast and pericycle protoplast. Deposited Si was present in the endodermal ITW, outer and radial wall, xylem parenchyma cell wall and peripheral metaxylem wall. The results in chapter 4 (see section 4.3.1) also demonstrated silica within the ITW of the endodermal cells and therefore the silica must be present in the apoplast at some point in time. This may be on its journey to the stele or possibly as a leakage out of the xylem (see section 3.1). When the plants were subjected to 0.5 mM silica the xylem concentration was about 3 mM Si (tab. 4.3). As the concentration within the xylem can exceed the concentration of the silica in the external medium a step in transport must concentrate the silica.

The current data suggest that the transport and uptake of silica is differentially inhibited by various ATPase inhibitors. This may be due to there being two pumps in operation (at the PM of endodermal and xylem parenchyma cells, see 5.1). Silica uptake and transport appeared to depend upon the activity of an ATPase. As the ionophores (nigericin and FCCP)

also inhibit silica uptake and transport, it is suggested that the transport is not a specific Si-ATPase pump, but is more likely to be driven by a proton gradient generated by a  $H^+$ -ATPase.

The suggested model proposes that the neutral silica species is symported with protons across the PM. This may occur in any cell prior to the endodermis. X-ray microanalysis has detected Si in the endodermal protoplast, but not in the cortical cells (Hodson & Sangster, 1989c), and the endodermis therefore appears to be the most likely site of Si uptake into the symplast (fig. 5.14).

It is possible that uptake of silica may not be *via* symport with protons. This is the most simple suggestion based upon the inhibitor studies presented (suggesting ATPases and a proton gradient is required). It is possible that Si could be symported/antiported with another ion which is moving down an electrochemical gradient established by the proton gradient. In diatoms it has been shown that sodium (Bhattacharyya & Volcani, 1980) and potassium gradients (Sullivan, 1976) are utilized. Present results with FCCP suggest a proton gradient is required in wheat for silica transport.

Once across the PM it is suggested that Si transport continues by both apoplastic and symplastic routes. Remaining in the symplast, the Si moves into the pericycle (detected here by Hodson & Sangster, 1989c). Some Si "leaks out" of the endodermal cells perhaps *via* non-specific ion channels or pumps into the apoplast or alternatively, apoplastic silica may be solely due to leakage from the xylem. Some or all the apoplastic silica is deposited within the ITW matrix, however, it is unclear how or why silica deposition occurs within these walls (reviewed in section 4.1). Apoplastic silica pathways to small xylem vessels in wheat root have been observed (Hodson & Sangster, 1989c).

It is unlikely that the Si is concentrated enough within the stele to flow passively into the xylem vessel to produce the Si concentration observed within the xylem sap (tab. 4.3). Reports have suggested the functioning of proton pumps at the xylem parenchyma cells (see section

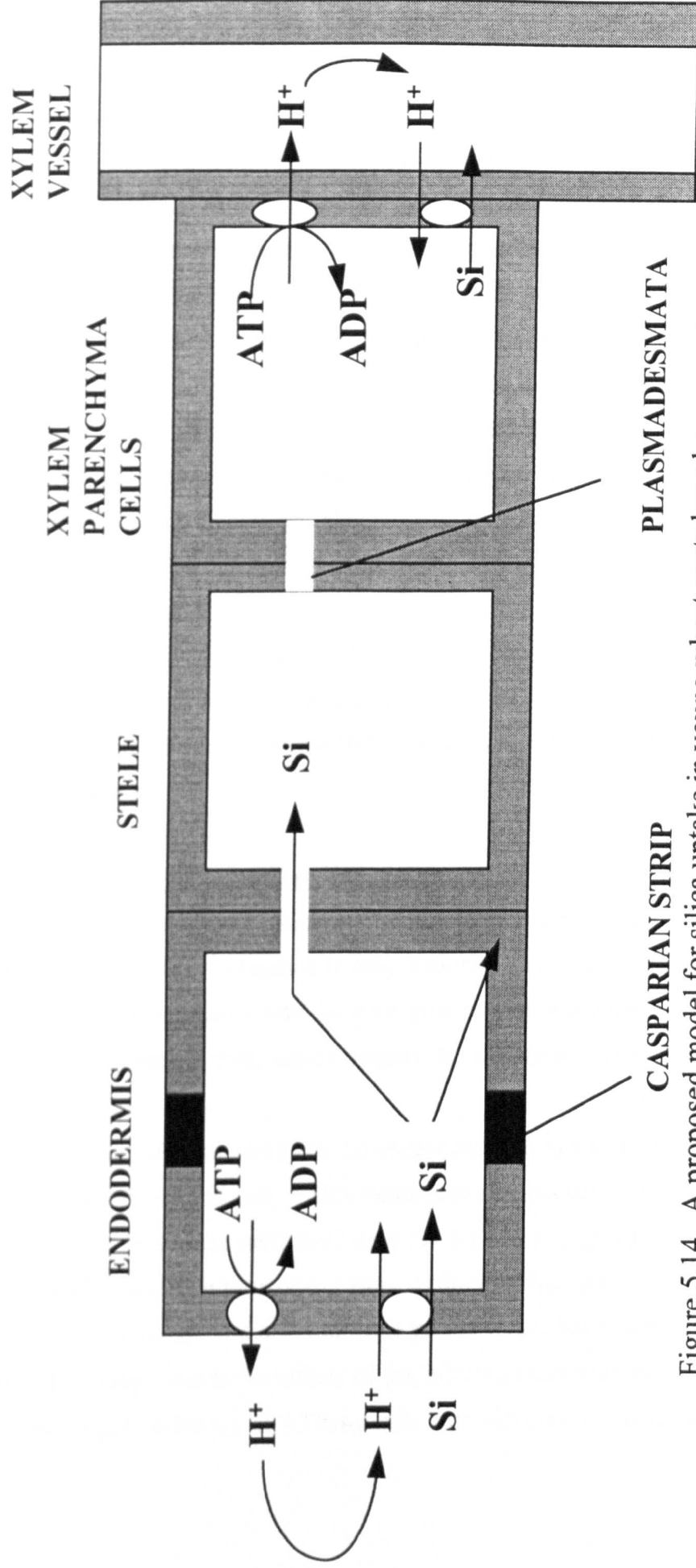


Figure 5.14. A proposed model for silica uptake in young wheat roots based on present experimental data.

5.1). In the present proposed model, it is suggested that the proton gradient generated is used to drive Si transport into the xylem vessel possibly *via* an antiport system with protons, in contrast to the symport system suggested for entering the symplasm. It is implied that Si is pumped directly into the xylem vessel rather than accumulating in the xylem parenchyma cells (XPC) and flowing down a Si concentration gradient as high Si concentrations have not been detected in the XPC. If Si is transported as a neutral species the proposed transport mechanisms would be electrogenic in nature.

A further possibility is that silica transport occurs within the root tip before the apoplastic pathway is blocked by the Casparian strips. The evidence to date does not support this idea as silica does not enter solely by mass diffusion (see section 5.3.4) and uptake is reduced by the inhibitors in the present study (section 5.3.5).

It is believed that this model is in keeping with current information available for Si uptake. It is very speculative and further work will no doubt alter the proposed model considerably, but, such a model is necessary to suggest further work.

#### Problems Associated With the Present Investigation.

The data from this investigation are by no means complete and it must be stressed that this investigation is only a preliminary study. Using whole seedlings as experimental material does give data on transport processes, however, excised roots would possibly be a simpler system to study.

The detection of silica using the molybdate reaction is a continuing difficulty, due to lack of sensitivity. This meant experiments had to be conducted over relatively long periods of time (24 h.) and at high silica concentrations (2 mM). The time of exposure to the inhibitor was considered to be a problem, and a shorter time period would have been preferable. Over long periods the effects of the inhibitors can become less specific as prolonged inhibition of ATPases will have obvious detrimental

effects on the physiology of the whole plant. Also, the inhibitors effects and the observed results may be far removed. For example, inhibiting the proton-ATPase causes a decline in the proton gradient, and this in turn reduces potassium uptake. The potassium deficiency induced would cause widespread disruption of the metabolism of the plant. This may then lead to a reduction in silica uptake. These "secondary effects" of the inhibitors would be reduced if the time course used was shorter.

The use of whole plants is also a problem because the exact site of inhibitor action within the tissue is unknown. It is also possible that the plant could inactivate the inhibitor, perhaps by transporting and sequestering within the root cell vacuole or transporting to the shoot.

The inhibitors effect on transpiration was not measured. If a large proportion of silica enters *via* mass flow and the inhibitor reduces transpiration this would appear to inhibit silica uptake. However, reports have suggested that silica uptake is not related to transpiration rates (see section 5.1 and 5.3.4).

#### 5.4.7. Further Investigations.

Obviously a great deal of work is needed to elucidate silica uptake and transport mechanisms in higher plants. It is suggested that further work be conducted using excised roots, and then more simplified systems such as protoplasts and microsomal vesicles. Short time courses might be used (as discussed above), but this would necessitate the use of  $^{31}\text{Si}$ . Work could be directed towards studying possible interactions between silica and other plant nutrients within the nutrient solution and in uptake studies. Further investigations should determine if silica is taken up uniformly along the whole root axis or if uptake is related to endodermal development, similar to the experiments concerning calcium (see Clarkson, 1988) which clearly demonstrated calcium uptake was restricted to the root apex where Casparian strips were undeveloped.

If silica uptake could be demonstrated in protoplasts from higher plants numerous further studies could be conducted. The present inhibitor

investigation could be repeated using protoplasts and the results compared. Electrophysiological studies could also be performed because if silica, as a neutral species, is symported with an ion the system would be electrogenic.

The possibilities are considerable, and would hopefully lead to further elucidation of the mechanisms of silica uptake and transport within higher plants. Once this has been established work could then be directed towards deposition mechanisms.



## CHAPTER 6: CONCLUSIONS.

### 6.1. General Conclusions.

The initial chapter of this thesis was concerned with the analysis of a particular phytolith, the papilla, from the inflorescence bracts of barley, wheat, wheat progenitors, and a preliminary investigation of rye and triticale. The parameters of pit number and papilla diameter were measured and forms the basis of a database. The results demonstrated that it is possible to distinguish between *Triticum aestivum* and *Hordeum vulgare* using papillae parameters only. It is also possible to discriminate between wheat with a different ploidy level using papilla parameters, although the extent of any genetic control could not be evaluated. A further observation was that new cultivars of the cereals studied had larger papilla parameters than older cultivars, suggesting cultivation possibly influences papilla parameters. Overlaps of parameters between the species were also highlighted.

Silica in the nutrient solution appeared to retard root growth of young wheat seedlings during the first 6 days after being transferred to the solution, when compared to plants which were grown in solutions without additional silica. However, after this initial period, root growth of plants supplied with silica increased such that at the termination of the experiment (10 days in total) these plants had significantly longer roots than plants without silica. The shoots of the plants did not appear to be as affected as the roots by the presence of silica.

A time course for the development of endodermal deposits within the root of wheat was elucidated over a 10 day period using microscopical techniques. Slight silicification of the inner tangential wall of endodermal cells could be detected in the apical region of roots, but the basal (i.e. nearest the caryopsis) area of the root had the greatest percentage of endodermal cells silicified at any time. X-ray microanalysis, however, failed to locate silica conclusively in the roots of plants grown for 24 hours in 0.5 mM Si nutrient solution. Chemical techniques were used to determine the silica partitioning over a 10 day time period, demonstrating

that the quantity of silica within plants increases over time, although at certain times, the plant was growing faster than it was accumulating silica. The amount of deposited silica far outweighed the amount of soluble silica, and it was also apparent that the soluble silica content was variable. The concentration of silica within the xylem was also calculated and was found to be very high, in the range of 3mM, although again this was variable.

The final chapter was aimed at elucidating the mechanism of silica uptake in wheat plants. The results suggested that uptake required ATP, as it was partially inhibited by the ATPase inhibitors used, and a proton gradient, as the uptake of silica was reduced in the presence of proton ionophores. The results also suggest that xylem loading was an active process, although possibly inhibited differentially from uptake. This investigation was a preliminary analysis of silica uptake and a basis for further work.

## 6.2. Further Work.

The database of papillae parameters needs to be extended as the current work showed a great deal of variation within species of cereal studied. Further species and accessions need to be analyzed and any overlaps identified. This data needs to be added to the accumulation of data concerning other phytoliths, so that the possible taxonomic and archaeological uses of phytoliths can be exploited.

The growth of young wheat seedlings appeared to be affected by silica present in the solution, but further analysis of the plants is required. The elemental composition of the plant should be analyzed as the effect of silica on growth may be due to enhancement of nutrient uptake. Also the effect of silica on cell division/expansion should be investigated.

Further work concerning the deposition of silica within the endodermis may include determining the various cell wall components present during silica deposition. Also the effect of environmental conditions on the deposition rate could be investigated, as could the effect of inhibitors of cell wall synthesis. Silica partitioning within the plant demonstrated that a large fraction was deposited and the effect of environmental factors on this fraction could be investigated, for example, the effect of a pathogenic attack on the plant. The use of radioactive silicon would be useful to monitor changes in partitioning over short time periods, although it has a short half life (see section 4.1). The xylem concentration of silica is very high, and further work could be directed towards determining if this silica is bound or is present as free silica.

The investigation into uptake mechanisms suggested possibilities for silica uptake in plants, but a great deal of further work is required in this particular area, and as was proposed in the discussion section of chapter 5.

# Appendices.

## APPENDIX I

**Table 1.** The average root length of plants grown in either full nutrient solution without silica (Si-) or full nutrient solution with 0.5 mM Si added (Si+) for 10 days.

Days	Average Root Length (mm)	
	Si+ plants	Si-plants
0	19.97	19.97
1	25.04	27.7
2	23.04	26.67
3	24.02	31.96
4	27.56	33.36
5	33.89	47.46
6	76.11	64.56
7	105.09	64.66
8	110.47	70.54
9	112.6	88.41
10	110.14	86.1

**Table 2.** The fresh weight of the roots and shoots of plants grown in either full nutrient solution without silica (Si-) or full nutrient solution with 0.5 mM Si added (Si+) for 10 days.

Days	Fresh Weight (mg) per plant			
	Si+ Roots	Si+ Shoots	Si- Roots	Si- Shoots
0	11.74	4.44	11.74	4.44
1	7.15	15.60	15.86	13.08
2	11.60	23.67	18.81	27.38
3	19.24	38.76	44.02	38.43
4	15.92	58.49	22.78	39.15
5	15.06	76.43	49.56	69.88
6	43.23	71.44	65.15	92.50
7	61.06	132.28	51.18	144.21
8	65.70	155.81	87.40	161.02
9	82.07	183.51	90.10	162.76
10	84.24	186.46	62.73	147.25

## APPENDIX. II.

All chemicals were obtained from Sigma Chemicals, Dorset, England.

### Ammonium Molybdate Solution.

7.5 g ammonium molybdate tetrahydrate was dissolved in ultra-pure water.

10 ml of 18N sulphuric acid was added and the solution diluted to 100 ml.

This was then stored in a plastic container covered with aluminium foil at 4°C, until required.

### Reducing Solution.

0.07 g sodium sulphite was dissolved in 1 ml ultra-pure water.

0.015 g 1-amino, 2-naphthol, 4-sulphonic acid was added to the solution and dissolved. 0.9 g sodium bisulphite was dissolved in 8 ml of distilled water and added to the solution. This was made up fresh in a plastic beaker which was covered in aluminium foil.

### APPENDIX III.

Below is an example of the calculations performed to produce the calculated data in chapter 4. Plants were grown for up to 10 days in full nutrient solution with 0.5 mM silica added. Using the raw data, it is possible to calculate soluble and deposited silica per root system, quantity of silica within the xylem vessel, the percentage contribution each silica pool (soluble, deposited and within the xylem) makes to the total silica and, finally, to calculate the concentration of silica within the "root water", i.e. within the apoplast and symplast, but excluding the xylem vessels.

#### Calculations for a 2 day old plant.

##### Raw Data (measured directly).

Average Mean Root Length = 23.04 mm (from appendix I, table 1)  
Root System Fresh Weight = 11.6 mg/plant (from appendix I, table 2)  
Xylem Diameter = 0.0625 mm (measured in sections prepared in 4.2.3)  
Total Silica = 1.564  $\mu\text{molesSi/gFWT}$  (table 4.3)  
Soluble Silica (including xylem) = 0.181  $\mu\text{molesSi/gFWT}$  (table 4.3)  
Silica Concentration within the Xylem = 2.07 mM (table 4.3)

##### Calculations

###### Silica Content per Root System:

Total Silica =  $1.564 \times 11.6/1000 = 1.814 \times 10^{-2} \mu\text{moles}$   
Soluble Silica =  $0.181 \times 11.6/1000 = 2.100 \times 10^{-3} \mu\text{moles}$   
THEREFORE: Deposited Silica =  $(1.814 \times 10^{-2}) - (2.100 \times 10^{-3})$   
 $= 1.604 \times 10^{-2} \mu\text{moles.}$

###### Silica Within the Xylem:

Volume of xylem vessel =  $\pi \times \text{radius}^2 \times \text{length}$   
 $= \pi \times (0.0625/2)^2 \times 23.04 = 7.069 \times 10^{-2} \text{ mm}^3$

THEREFORE: the vessel contains  $7.069 \times 10^{-5} \text{ cm}^3$ . ( $1 \text{ cm}^3 = 1 \text{ ml}$ )

The silica concentration in xylem = 2.07 mM

Silica  $\mu\text{moles}$  in Xylem =  $2.07 \times (7.069 \times 10^{-5}/1000) \times 1000$   
 $= 1.463 \times 10^{-4} \mu\text{moles Silica}$

THEREFORE: Assuming each plant had 3 roots of average length and that each root had one xylem vessel, the number of  $\mu\text{moles}$  in a root system =

$3 \times (1.463 \times 10^{-4}) \mu\text{moles} = 4.389 \times 10^{-4} \mu\text{moles in the xylem}$

In a 2 Day Root System:

$$\text{Total Silica} = 1.814 \times 10^{-2} \mu\text{moles}$$

$$\text{Xylem Silica} = 4.389 \times 10^{-4} \mu\text{moles}$$

$$\begin{aligned}\text{Soluble Silica} &= (0.210 \times 10^{-2}) - (4.389 \times 10^{-4}) \\ &= 1.661 \times 10^{-3} \mu\text{moles}\end{aligned}$$

$$\text{Deposited Silica} = 1.604 \times 10^{-2} \mu\text{moles}$$

Percentage of Total Silica:

$$\text{Xylem} = (4.389 \times 10^{-4} / 1.814 \times 10^{-2}) \times 100 = 2.4\%$$

$$\text{Soluble} = (1.661 \times 10^{-3} / 1.814 \times 10^{-2}) \times 100 = 9.16\%$$

$$\text{Deposited} = (1.604 \times 10^{-2} / 1.816 \times 10^{-2}) = 88.42\%$$

Concentration of Silica in "Root Water" Excluding the Xylem.

$$\text{Amount of H}_2\text{O in root system} = \text{FWT} - \text{DWT}$$

$$= 11.6 - 0.83 \text{ mg} = 10.77 \times 10^{-3} \text{ g}$$

$$1 \text{ ml H}_2\text{O} = 1 \text{ g}$$

THEREFORE: The root contains  $10.77 \times 10^{-3} \text{ ml}$  of  $\text{H}_2\text{O}$

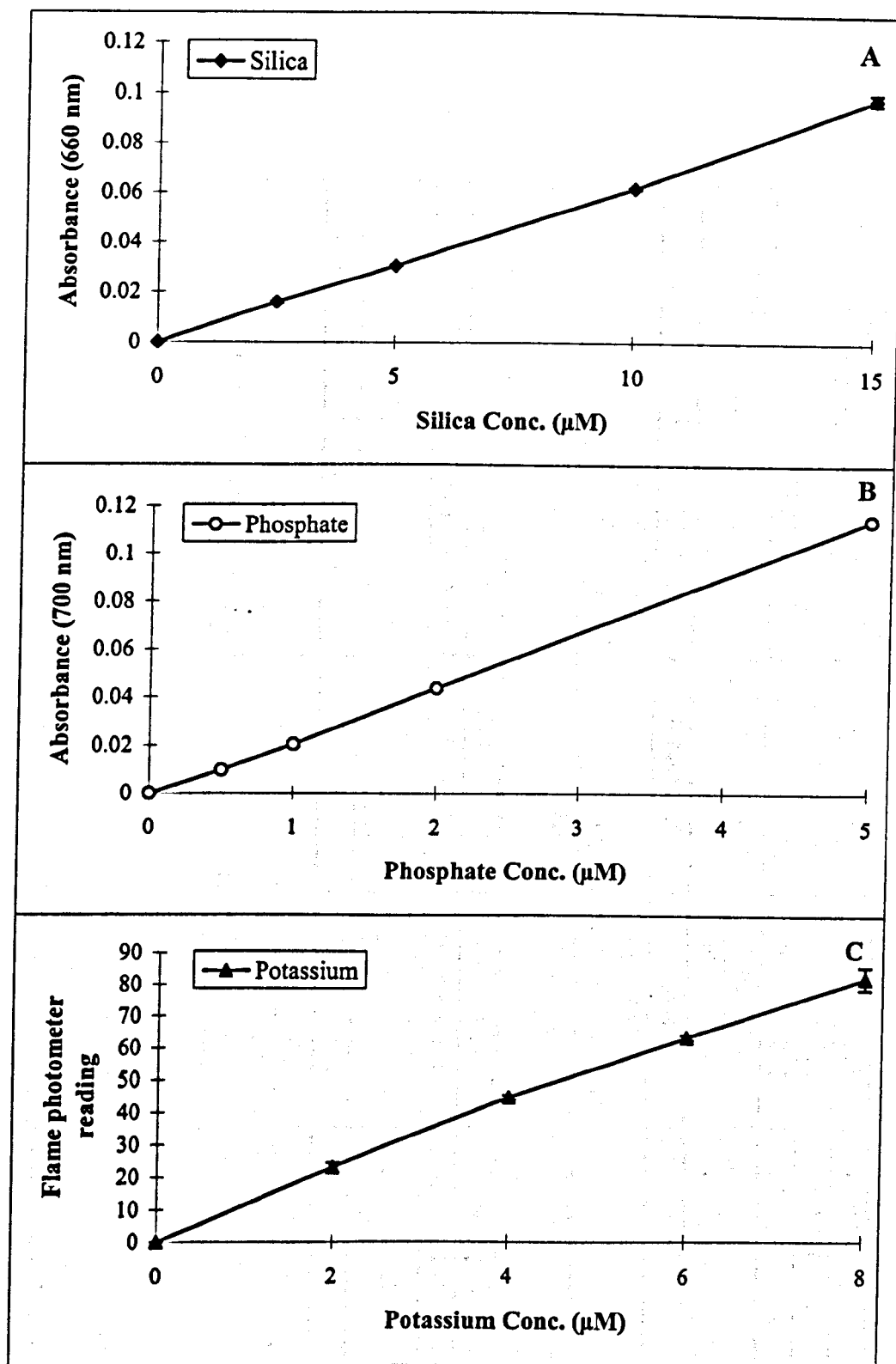
in the xylem there is  $7.069 \times 10^{-5} \text{ ml}$

$$\text{Excluding the xylem} = 10.77 \times 10^{-3} - (7.069 \times 10^{-5} \times 3) = 1.056 \times 10^{-2} \text{ ml}$$

This volume contains  $1.661 \times 10^{-3} \mu\text{moles}$  silica, therefore the concentration =

$$1.661 \times 10^{-3} \times 1000 / (1.056 \times 10^{-2}) = 33.144 \mu\text{m}$$





Standard curves for silica, phosphate and potassium analyses. Samples prepared in section 5.2.6. were compared to these graphs,  $n=2 \pm \text{std.}$  (in many cases std. is less than the size of the data symbol and thus obscured).

A) Silica, absorbance measured at 660 nm.

B) Phosphate, absorbance measured at 700 nm.

C) Potassium, readings from a flame photometer using a potassium filter.

**Table 1.** The silica, potassium and phosphate content of wheat plants grown in nutrient solution in the presence of sodium orthovanadate (vanadate) for 24 hours. Si<sup>+</sup> plants were grown in nutrient solution containing 2 mM Si. Si<sup>-</sup> plants were grown in nutrient solution without added silica. n=4.

Roots						
Vanadate Conc. ( $\mu\text{M}$ )	Si+ Plants ( $\mu\text{moles/gDWT}$ ) (mean $\pm$ std.)			Si- Plants ( $\mu\text{moles/gDWT}$ ) (mean $\pm$ std.)		
	Silica	Potassium	Phosphate	Potassium	Phosphate	Phosphate
0	18.38 $\pm$ 6.6	594.8 $\pm$ 88.2	160.9 $\pm$ 38.5	740.1 $\pm$ 61.5		122.7 $\pm$ 31.2
25	19.41 $\pm$ 0.99	505.7 $\pm$ 119.2	125.3 $\pm$ 21.6	514.5 $\pm$ 69.4		117.5 $\pm$ 24.4
50	22.47 $\pm$ 9.6	440.7 $\pm$ 97.7	111.1 $\pm$ 19.9	386.0 $\pm$ 35.0		102.6 $\pm$ 7.0
75	20.54 $\pm$ 10.9	416.4 $\pm$ 47.3	111.5 $\pm$ 9.4	463.0 $\pm$ 123.2		136.6 $\pm$ 28.1
100	10.84 $\pm$ 4.41	494.4 $\pm$ 96.6	128.4 $\pm$ 16.5	443.0 $\pm$ 57.0		141.6 $\pm$ 49.0

Vanadate Conc. ( $\mu\text{M}$ )	Shoots Si+ Plants ( $\mu\text{moles/gDWT}$ ) (mean $\pm$ std.)			Si- Plants ( $\mu\text{moles/gDWT}$ ) (mean $\pm$ std.)	
	Silica	Potassium	Phosphate	Potassium	Phosphate
0	7.905 $\pm$ 5.264	285.5 $\pm$ 99.4	159.58 $\pm$ 9.205	332.2 $\pm$ 38.8	157.01 $\pm$ 18.67
25	12.402 $\pm$ 8.587	316.7 $\pm$ 147.1	163.99 $\pm$ 48.51	246.6 $\pm$ 77.9	151.90 $\pm$ 13.47
50	8.696 $\pm$ 2.977	262.5 $\pm$ 40.6	142.23 $\pm$ 25.92	269.5 $\pm$ 74.8	148.23 $\pm$ 8.79
75	18.043 $\pm$ 0.43	213.4 $\pm$ 29.5	132.04 $\pm$ 2.21	252.9 $\pm$ 97.9	144.90 $\pm$ 29.73
100	5.164 $\pm$ 3.864	212.1 $\pm$ 76.6	122.07 $\pm$ 18.67	300.2 $\pm$ 122.2	168.90 $\pm$ 37.74

**Table 2.** The silica, potassium and phosphate content of wheat plants grown in nutrient solution in the presence of erythrosin B for 24 hours. Si+ plants were grown in nutrient solution containing 2 mM Si. Si- plants were grown in nutrient solution without added silica. n=4.

Erythrosin B Conc. (µM)	Roots			
	Si+ Plants (µmoles/gDWT) (mean ± std.)			Si- Plants (µmoles/gDWT) (mean ± std.)
	Silica	Potassium	Phosphate	Phosphate
0	23.99 ± 9.12	450.1 ± 108.5	112.0 ± 11.5	451.3 ± 80.4
0.5	22.97 ± 4.73	451.0 ± 73.8	124.5 ± 20.6	469.8 ± 126.2
1	31.02 ± 5.51	427.8 ± 89.4	113.5 ± 5.9	459.0 ± 96.1
5	23.28 ± 2.93	550.4 ± 40.9	124.9 ± 13.8	533.3 ± 99.7
10	16.18 ± 0.58	529.1 ± 66.1	112.0 ± 20.8	583.7 ± 157.5
				133.1 ± 14.3

Erythrosin B Conc. (µM)	Shoots			
	Si+ Plants (µmoles/gDWT) (mean ± std.)			Si- Plants (µmoles/gDWT) (mean ± std.)
	Silica	Potassium	Phosphate	Phosphate
0	11.27 ± 3.62	434.5 ± 26.57	157.52 ± 10.14	433.8 ± 29.19
0.5	6.84 ± 3.04	389.5 ± 21.94	156.58 ± 8.15	413.7 ± 42.32
1	7.58 ± 2.88	363.4 ± 36.61	139.89 ± 17.47	392.3 ± 45.20
5	5.91 ± 1.95	419.8 ± 81.51	141.24 ± 15.62	423.5 ± 59.54
10	4.75 ± 0.62	421.0 ± 17.2	133.72 ± 12.10	425.0 ± 9.53
				158.34 ± 14.08
				154.47 ± 13.48
				165.28 ± 13.74
				145.03 ± 12.52
				139.40 ± 8.19

**Table 3.** The silica, potassium and phosphate content of wheat plants grown in nutrient solution in the presence of diethylstilbestrol (DES) for 24 hours. Si+ plants were grown in nutrient solution containing 2 mM Si. Si- plants were grown in nutrient solution without added silica. n=4.

DES Conc. (µM)	Roots					
	Si+ Plants (µmoles/gDWT) (mean ± std.)			Si- Plants (µmoles/gDWT) (mean ± std.)		
	Silica	Potassium	Phosphate	Potassium	Phosphate	
0	17.69 ± 1.80	358.33 ± 60.6	106.73 ± 9.90	362.9 ± 28.9	116.08 ± 21.46	
25	30.21 ± 7.65	325.50 ± 79.4	104.23 ± 16.46	330.1 ± 27.2	101.80 ± 9.27	
50	24.63 ± 2.15	295.10 ± 27.2	100.24 ± 11.66	327.4 ± 33.7	97.79 ± 8.09	
75	26.82 ± 10.38	306.30 ± 66.8	99.22 ± 15.59	303.2 ± 21.6	104.44 ± 10.87	
100	13.76 ± 6.63	314.10 ± 43.1	101.53 ± 23.22	324.2 ± 44.3	106.42 ± 18.18	

DES Conc. (µM)	Shoots					
	Si+ Plants (µmoles/gDWT) (mean ± std.)			Si- Plants (µmoles/gDWT) (mean ± std.)		
	Silica	Potassium	Phosphate	Potassium	Phosphate	
0	11.72 ± 1.81	375.90 ± 47.72	168.54 ± 27.73	374.08 ± 30.33	164.20 ± 23.24	
25	9.00 ± 1.93	349.95 ± 47.02	141.35 ± 13.60	357.15 ± 40.45	140.88 ± 12.57	
50	9.08 ± 2.68	338.65 ± 47.21	144.90 ± 8.22	304.65 ± 7.42*	137.14 ± 19.52	
75	9.34 ± 1.40	358.95 ± 35.78	137.77 ± 16.06	360.08 ± 51.90	146.72 ± 12.38	
100	4.45 ± 2.91	337.1 ± 27.22	131.04 ± 6.45	353.80 ± 32.78	140.37 ± 9.48	

\* n=2

**Table 4.** The silica, potassium and phosphate content of wheat plants grown in nutrient solution in the presence of nigericin for 24 hours. Si+ plants were grown in nutrient solution containing 2 mM Si. Si- plants were grown in nutrient solution without added silica. n=4.

Nigericin Conc. (μM)	Roots					
	Si+ Plants (μmoles/gDWT) (mean ± std.)			Si- Plants (μmoles/gDWT) (mean ± std.)		
	Silica	Potassium	Phosphate	Potassium	Phosphate	
0	38.56 ± 8.19	387.5 ± 37.9	96.05 ± 7.55	442.5 ± 35.7	101.07 ± 17.72	
0.3	22.22 ± 8.07	428.2 ± 36.7	103.31 ± 8.14	424.3 ± 83.9	89.56 ± 19.52	
0.6	30.23 ± 12.58	390.2 ± 48.5	91.98 ± 10.88	381.9 ± 42.4	85.52 ± 8.92	
1	21.00 ± 11.21	358.9 ± 70.3	82.67 ± 20.76	427.6 ± 56.8	99.77 ± 7.46	
10	26.13 ± 10.50	430.6 ± 70.4	88.34 ± 8.28	417.2 ± 27.8	84.5 ± 16.87	

Nigericin Conc. (μM)	Shoots					
	Si+ Plants (μmoles/gDWT) (mean ± std.)			Si- Plants (μmoles/gDWT) (mean ± std.)		
	Silica	Potassium	Phosphate	Potassium	Phosphate	
0	7.10 ± 2.916	410.15 ± 43.27	158.66 ± 10.13	415.95 ± 69.78	162.77 ± 14.61	
0.3	8.94 ± 0.891	376.38 ± 146.78	153.72 ± 11.21	305.25 ± 51.57	143.66 ± 14.00	
0.6	6.12 ± 2.565	355.70 ± 117.15	150.84 ± 11.76	356.98 ± 53.67	160.59 ± 6.41	
1	5.58 ± 2.284	383.20 ± 74.88	143.08 ± 18.08	356.08 ± 81.82	154.59 ± 11.77	
10	6.01 ± 2.555	394.80 ± 35.05	137.55 ± 19.64	328.65 ± 62.41	148.29 ± 18.35	

**Table 5.** The silica, potassium and phosphate content of wheat plants grown in nutrient solution in the presence of FCCP for 24 hours. Si+ plants were grown in nutrient solution containing 2 mM Si. Si- plants were grown in nutrient solution without added silica. n=4.

FCCP	Roots			
	Si+ Plants (μmoles/gDWT)			Si- Plants (μmoles/gDWT)
	Silica	Potassium	Phosphate	
Conc. (μM)		(mean ± std.)		(mean ± std.)
0	22.06 ± 7.94	372.4 ± 58.0	125.42 ± 16.75	Potassium 430.3 ± 38.9 Phosphate 144.20 ± 22.9
25	44.81 ± 14.76	303.0 ± 45.6	114.95 ± 18.06	290.2 ± 24.4 95.86 ± 17.40
75	25.00 ± 10.80*	251.8 ± 125.4	91.90 ± 29.50	264.4 ± 41.2 92.70 ± 19.26
100	23.78 ± 1.80	246.6 ± 17.7	866.74 ± 13.34	319.7 ± 99.5 117.18 ± 16.26

FCCP	Shoots			
	Si+ Plants (μmoles/gDWT)			Si- Plants (μmoles/gDWT)
	Silica	Potassium	Phosphate	
Conc. (μM)		(mean ± std.)		(mean ± std.)
0	12.46 ± 2.249	538.1 ± 68.4	175.24 ± 16.01	Potassium 438.8 ± 33.3 Phosphate 169.11 ± 26.89
25	5.19 ± 1.771	442.2 ± 70.3	165.86 ± 17.42	481.2 ± 11.8 182.00 ± 31.81
75	6.97 ± 1.958	383.6 ± 76.7	154.57 ± 15.03	450.4 ± 24.9 168.99 ± 16.57
100	7.39 ± 2.289	412.7 ± 75.7	158.96 ± 10.73	441.0 ± 25.8 172.33 ± 17.55

\*n=3

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