SILICON UPTAKE IN RICE AND CUCUMBERS

by

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ABSTRACT

The study of silicon uptake in rice (*Oryza sativa* L. cv. M102) revealed an energy dependent, carrier-mediated system of silicon transport. This uptake system saturated at approximately 2 mM external Si and displayed a V_{max} of 18.5 μmol.g root^{-1}.h^{-1} and a K_m of 0.578 mM. Transport was found to not rely on transpiration rates. Studies of the effect of temperature, anoxia and KCN showed that the silicon uptake system was as strongly dependent on metabolic energy as potassium and several other major macronutrients, though it responded more slowly to such treatments than did potassium. The uptake system showed the characteristics of a derepressible system of uptake, in which plants deprived of silicon showed markedly enhanced rates of uptake as compared with plants grown in the presence of adequate silicon levels.

The study of the kinetics of uptake in cucumbers (*Cucumis sativus* cv. Corona) revealed the possibility that, rather than passively absorbing silicon, the plants possess a low affinity saturable system responsible for the uptake of silicon, with a K_m of 0.84 mM and a V_{max} of 12.5 μmol.g root^{-1}.h^{-1}. This system, however, did not appear to have a derepressible system of uptake such as was the case for rice.

In the light of the growing body of information pointing to the importance of silicon as a plant nutrient, this study provides physiological evidence of silicon accumulation by two plant species and lays the foundation for fuller physiological and biochemical studies which may resolve the question of the essentiality of this element in plants.

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Temp = 25°C, RH = 60%, LI = 500 μE.m^{-2}.s^{-1}
Initial [Si] = 1 mM
3 replicates represented by lines A(——), B(----), and C(---)
Samples every 1 h for 30 h.

Temp = 25°C, RH = 60%, LI = 500 μE.m^{-2}.s^{-1}
Initial external [Si] = 0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, 1.0, 1.5, 20., 3.0 mM
Samples every 15 min for 105 min.
Error bars represent 95% confidence limits.
Line represents a Michaelis Menten fit as analyzed kinetically in figure 4

Data used are those represented in Figure 3.
V represents uptake (μmol.g^{-1}.h^{-1}.) [S] represents substrate concentration (mM).
r^2 = 0.936, K_m = 0.587 mM, V_{max} = 18.5 μmol.g^{-1}.h^{-1}

Temp = 25°C, LI ~ 500 μE.m^{-2}.s^{-1}
Low RH=50%, Medium RH~70-80%, High RH=100%
Initial [Si] = 0.5 mM Si
Samples every 40 min for 200 min.
Error bars represent 95% confidence limits.

Expected Uptake = Volume lost to transpiration × External [Si]
Actual uptake reflects data from Figure 5.

RH = 60%, LI = 500 μE.m^{-2}.s^{-1}
Temp. = 10, 15, 20, 25, 30 ° C
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
Samples every 30 min for 3 h.
Error bars represent 95% confidence limits.

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
Samples every 30 min for 3 h
Error bars represent 95% confidence limits.
Percentages with each series represent the change in transpiration or uptake upon the imposition of anoxic conditions. The arrows accompanying those percentages represent the direction of this change.

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
Samples every 30 min for 3 h
Error bars represent 95% confidence limits.

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
Samples every 30 min for 3 h
Error bars represent 95% confidence limits.

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
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Error bars represent 95% confidence limits.

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$
Initial [Si] = 1 mM
Samples taken every 4 h for 32 h

LEGENDS TO THE TABLES

RH = 60%, LI = 500 μE.m^{-2}.s^{-1}
Temp. = 10, 15, 20, 25, 30 º C
Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM
Samples every 30 min for 3 h.

Q_{10} values were calculated as $(U_2/U_1)^{10/t_2 - t_1}$, where U_1 and U_2 are the uptake

rates at temperatures t_1 and t_2

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INTRODUCTION

Silicon is the second most abundant element in the earth's crust (Raven, 1983), making up 27.6 atomic percent of its mineral content (Jackson, 1964). In combination with oxygen, it forms the framework of soil. It is leached out of the soil by the processes of weathering, and exists in the Earth's waters as monosilicic acid, $Si(OH)_4$. The average monosilicic acid concentration in the soil solution is 0.5 mM, but ranges from 0.01 to 1.3 mM, depending on soil pH, weathering conditions and soil parent material (Birchall, 1978). In seawater, its concentration ranges from 0-100 μM, and in freshwater bodies, from 0.4 to 180 μM, the lower concentrations being largely due to diatom depletion (Raven 1983).

The involvement of silicon in biological systems is best understood in protists and least understood in more highly evolved groups, such as higher plants and animals. Due to its ubiquity, as well as its uncharged nature and complex chemistry, a biological role for silicon, except in a few isolated cases such as diatoms and horsetails, has been generally regarded as unlikely. However, as more information accumulates, silicon is emerging as a vital, though poorly understood, nutrient for plants and animals, for which further studies examining its physiology and biochemistry in such groups is now imperative.

I. PHYSICAL PROPERTIES OF BIOLOGICAL SILICON

In the physiological range (pH 2-9), monosilicic acid is electroneutral. Above 9, it begins to ionize (pK_1=9.82) and its solubility rises sharply, encouraging the dissolution of polymerized or colloidal forms of silica. At pH 5.5 to 6 conditions are favorable for the formation of dimers, trimers, and eventually polysilicic acid (Barber and Shone, 1966; Lewin and Reiman, 1969; Aston and Jones, 1976). Silicon chemistry in water is limited due to the extreme stability of Si-O-Si units, which favors the formation of condensed phases, be they amorphous or crystalline. Studies have shown that solutions greater than 5mM $Si(OH)_4$ polymerize to form colloidal silica or silica gel (Alexander et al., 1954). As

the kinetic energy required for crystallization is very high, the form of silicon generally found in biological systems, where temperature and pressure are low, is the amorphous gel phase. This can include anything from ordered opaline aggregates to hydrated gel-like deposits, though all will have the basic formula $[SiO_{n/2}(OH_{4-n})]_m$, where n=0 to 4 and m is a large number. The bond angle Si-O-Si in amorphous silica can assume a wide variety of values, as can the stoichiometry of the substance. Its nature is therefore highly variable and strongly influenced by the cellular processes surrounding its formation. At the molecular level, biogenic silica shows no sign of regular ordering and appears to be a random network of SiO_4 units, perhaps with certain elements such as S, Cl, and K distributed throughout. However, at the microscopic level morphological structure begins to become apparent. The two basic types of units are gel-like and particulate, which are differentiated by the degree of aggregation of colloidal particles during the silicification process. Above pH 5, polysilicic acid colloids are negatively charged due to the acid nature of their surface hydroxyl groups. Cations bind to these negatively charged surfaces, reducing repulsive forces between the particles and encouraging the formation of the gel-like phase. Under acidic conditions, repulsive forces between the particles are stronger, and the particulate phase results. These two phases can, in turn, assume many structural motifs, ranging from sheet-like, to globular, to fibrillar. In plant trichomes, these siliceous structures are thought to develop as small silica particles (1-5 nm) from a supersaturated silicic acid solution and are aligned on the surface of some organic "template" component. The structure then grows from the aggregation of particles at its surface (Mann and Perry, 1986). Silicon deposits in plant roots are formed as gel-like particles, deposited as a gel and bond to the cellulose fibers of the cell wall. This reaction is also favored by the presence of organic polar molecules, with which silicic acid can associate through hydrogen bonding. Silanol groups, on the surface of colloidal silica, react strongly with basic metal hydroxides, which is the suggested mechanism for the strong adsorption of

silicic acid by iron and aluminum oxides. They also react with hydroxy compounds to form esters (Birchall, 1978).

II. INVOLVEMENT OF SILICON IN LIVING SYSTEMS

In the introduction to "Silicon and Siliceous Structures in Biological Systems" (Simpson and Volcani, 1981), Volcani states that, "The occurrence of silica in 'primitive' (protistan) organisms and the essential involvement of silicic acid in biochemical and growth processes in these forms, as well as in other more highly evolved groups, suggest that early life forms may have been dependent upon this element, a dependence that has been carried through evolution to present day organisms." The essential nature of silicon in diatoms is the most fully understood role of the element in living systems, and other members of the Protista have also been shown to depend on it. As one moves to more highly evolved forms of life, the role of silicon is less well understood, though several groups in Animalia, as well as selected members of Plantae, have demonstrated a dependence on silicon as well. In Plantae, consistent with Volcani's statement, the more primitive vascular plants, ferns and horsetails, are those which contain the most silicon, though the role the element may play in them has not been examined (Takahashi et al., 1990). Higher plants contain variable amounts of silicon, and though several possible roles for it have been suggested, its biochemical, metabolic and physiological behavior has yet to be determined. Epstein (1994) states that ample evidence now exists that silicon, when available to plants, "plays a large role in their growth, mineral nutrition, mechanical strength and resistance to fungal diseases, herbivory, and adverse chemical conditions of the medium" and he suggests that plants grown hydroponically without added silicon are experimental artifacts. He has further remarked that (1991), "There seem to have been no physiological studies, such as those with diatoms, that would throw light on the biochemical events resulting in (...) silica depositions in higher plants."

The goal of this thesis is to undertake such studies, based upon nutritional studies in higher plants as well as earlier work with organisms in which silicon is more fully understood; information regarding silicon in these systems is invaluable in attempting to investigate the possibility of a similar role for silicon in plants.

A. DIATOMS

i. Essentiality of silicon in diatoms

In diatoms, silicon is well established as an essential element. They have the ability to accumulate silicon from very low concentrations in the environment and construct a siliceous cell wall, or frustule. This structure is composed of a matrix of silica polymers and organosilicates. Silicon absorbed from the environment is transformed in the Golgi apparatus and endoplasmic reticulum to form the wall materials, which are then transported in membrane-bound vesicles to the site of frustule formation. This transport is microtubule directed, and the vesicle contents are deposited on a membranous template, the silicalemma. This process is particularly active during cell division, and terminates with the discontinuation of silicon deposition, the coating of the frustule with an organic matrix, and the separation of the daughter cells (Birchall 1978; Sullivan 1986). When diatoms are transferred to silicon-free medium, cell division ceases within one hour, due to the inability of the cells to form frustules in the absence of silica. Cellular functions such as the syntheses of proteins, nucleic acids and chlorophyll also cease during silicon deprivation (Lewin and Reimann, 1969). In *Navicula pelliculosa*, a freshwater diatom, synthesis of DNA, RNA, protein and chlorophyll ceased within 7 hours in a silicon-free medium (Darley and Volcani, 1969; Lewin and Reimann, 1969; Coombs et al., 1967b). In addition, decreased rates of cellular metabolism and photosynthesis are observed in silicon-starved diatom cultures, which return to normal when adequate levels of silicon are resupplied. Coombs and Volcani (1968) examined the recovery of silicon starved cells following the addition of silicon. They found that amino acid production as well as protein

synthesis decreased during starvation, but increased immediately upon silicon resupply. Increased incorporation of ^{14}C and ^{32}P into intermediates of the TCA cycle and processes of photoreduction, respectively, were also observed in cells that had been resupplied with silicon after a period of starvation, indicating an increase in cell metabolic and photosynthetic functions in the presence of silicon. Incorporation of these isotopes into lipids, which increased during starvation, decreased when cells were returned to silicon-enriched medium. Cell division, which had ceased in the absence of silicon, resumed within seven hours of silicon being reintroduced. The decay of cellular functions in the absence of silicon, and their return to normal as silicon is restored, indicates the involvement of silicon in major metabolic pathways in diatoms, though the mechanism of such involvement is still unclear.

ii. Silicon uptake in diatoms

Studies have confirmed the existence of a carrier-mediated, energy-dependent transport system for the uptake of silicic acid by diatoms, which is characterized by both a high and a low affinity system (Sullivan, 1986). Studies by Azam et al.(1974) have demonstrated that $Si(OH)_4$ uptake in *Nitzschia alba* is temperature sensitive, showing a Q_{10} of ~ 2 at 20-30° C, while only minimal uptake was observed at 0° C. The system also displayed a pattern of uptake that could be described by Michaelis Menten kinetics, with a K_m of 4.5×10^{-6} M and a V_{max} of 3.35 $\mu mol.g^{-1}.h^{-1}$. On the basis of experiments using $Ge(OH)_4$ as an analog for $Si(OH)_4$ that is not metabolized, they concluded that *N. alba* expends metabolic energy to transport Si by both a high affinity and a low affinity system. These studies provided the foundation for further characterization and isolation of the Si transporter.

Marine diatoms have been more extensively studied than freshwater diatoms, and their silicon transport system has been determined to be a Na^+/Si symport; this may not be the case for freshwater diatoms. It was found that silicon transport in *Nitzschia alba* was

dependent on the presence of Na^+ and K^+ in the medium, and that the plasmalemma of the organism contained a Na^+/K^+ ATPase (Sullivan and Volcani, 1974). Battacharyya and Volcani (1980) subsequently found that silicon transport did indeed depend on the activity of the Na^+/K^+ ATPase, but could also be driven by an artificially imposed Na^+ gradient in isolated vesicles. By studying the effect of ionophores, they were able to determine that uptake was directly coupled with the Na^+ gradient rather than to a cation gradient in general. The model for silicon transport that emerged from these studies is as follows: the Na^+/K^+ ATPase forms an inwardly directed Na^+ gradient that provides the energy, by way of a Na^+/Si symport with a 1:1 stoichiometry, to transport silicon into the cell (Sullivan 1986).

The activity of the transport system seems to be linked to the cell cycle, as silicon uptake increases ten-fold prior to cell division. This rise in transport rates required *de novo* protein synthesis (Sullivan 1976b). Recent work has also shown that the transporter is unstable and that an increase in protein synthesis immediately prior to cell division corresponds to an increase in transporter synthesis. Once division has taken place, protein synthesis slows and, as more transporters are degraded than made, silicon uptake is also reduced (Sullivan 1986).

In their studies on the effect of nutrient starvation on uptake rates in *Thalassiosira pseudonana*, Parslow et al. (1984) found that Si-starved cultures had initially enhanced uptake rates which decreased following exposure to Si . These results are consistent with results using other species of diatoms (Conway et al., 1977). They would seem to indicate that the Si transport system is derepressible, similar to the derepressible uptake systems which have been characterized in higher plants for potassium (Glass, 1976; Siddiqi and Glass, 1986) and ammonium transport (Glass, 1988) .

B. OTHER ALGAE AND BACTERIA

Lewin and Reimann (1969) have reported that silicon is required for growth of several species of algae besides diatoms. Work by Moore and Traquair (1976) with the green alga *Cladophora glomerata* has shown silicon to be required for optimal growth in this species. In the absence of silicon, abnormal cell wall formation resulted in reduced growth of the algal filaments. In addition, they found that the addition of germanium, an analog of silicon, to the growth medium reduced the silicon level within the cell interior as well as within the cell wall, and substantially decreased the rate of growth of the algal filaments. Bacteria such as *Proteus mirabilis* take up monosilicic acid by an energy-dependent process, and are believed to secrete compounds capable of causing the breakdown of polysilicic acid to the monomeric form (Birchall, 1978).

C. ANIMALS

Silicon is considered essential in mammals and birds. Here it functions as a cross-linking agent in connective tissue, where it is required for proper tissue development. It is found as a constituent of certain glycosaminoglycans and polyuronides where it is bound to the polysaccharide matrix and to certain proteins in connective tissue such as collagen and elastin. It is also thought to be associated with calcium in the early stages of bone calcification.

The estimated nutritional requirement for chicks was 26-52 mg Si/1000 calories. Silicon deprivation in chicks and rats resulted in aberrant connective tissue and bone metabolism. The requirement for humans is thought to be 21-46 mg Si /day. It has been suggested, due to its importance in the development and maintenance of connective tissue and the observed decreases in silicon in aging humans, that lack of silicon may promote such disorders as hypertension, atherosclerosis and osteoarthritis (Parry et al., 1984).

The role of silicon in soft tissue is unknown. The cell nucleus has been found to contain a higher level of silicon than the mitochondria or rough endoplasmic reticulum,

which has led to the suggestion that silicon may have a role in the nucleus. Silicon has also been found at high levels in centrioles, suggesting the possibility of a role for silicon in their morphogenesis or function (Neilsen, 1982). The method by which animal cells absorb silicon has yet to be studied.

D. HIGHER PLANTS

Although silicon is not deemed essential for all plants, it is considered essential for certain plants, such as *Equisetum* spp., beneficial for others, such as rice and sugarcane, and deposits have been found in all plant species studied. In their survey of silicon in the plant kingdom, Takahashi et al. (1990) classified plants either as strong accumulators (Si content >5%, Si/Ca ratio >5), accumulators (Si content >2%, Si/Ca ratio >2), or non-accumulators (Si content <0.5%, Si/Ca ratio <0.5), of silicon. The strong accumulators included liverworts, certain families of ferns, and the tribes *Oryzoideae* and *Bambusoideae* of *Poaceae*. Accumulators included the remaining members of *Poaceae* and *Cyperaceae*, as well as certain mosses, ferns and some members of *Dicotyledoneae*. Non-accumulators were gymnosperms and the majority of members of *Dicotyledoneae*.

i. Effects of silicon on higher plant growth

Silicon deficiency symptoms in plants classed as accumulators of silicon range from necrosis of branch tips and collapse of plant stems in *Equisetum* species (Chen and Lewin, 1969), to drooping leaves and reduced reproductive and vegetative yield in members of Poaceae (Okuda and Takahashi, 1962a; Takahashi et al., 1990). Yields of barley, wheat, oats and rye were all found to be significantly decreased in the absence of silicon (Vlamis and Williams, 1967). In various grasses, silicon has been shown to play an important structural role; adding rigidity and tensile strength to the cell wall and tissues. It improves resistance to lodging, promotes an erect leaf habit that allows for better penetration of light and CO_2 into the community, and prevents excessive water loss from the leaf surface (Takahashi et al., 1990). Silicon appears to interact with the polyphenols

in xylem cell walls and may be involved in their metabolism and deposition (Parry and Kelso, 1975). It may affect lignin biosynthesis and deposition. Raven (1983) has suggested that silicon is as effective as a compression-resistant structural component of cell walls as is lignin, while the energetic cost of incorporating lignin per unit weight may be as much as 20 times higher. Reports of silicon deficiency symptoms in non-accumulator plants have been contradictory, and such symptoms have often been ascribed to improper nutrient balance in the growth medium (Takahashi et al., 1990; Marschner et al., 1990). However, Miyake and Takahashi have examined the effect of silicon deprivation on a variety of crop plants classed as non-accumulators. In all the non-accumulator species studied, deficiency symptoms appeared only after the first bud flowering stage. In tomato (Miyake and Takahashi, 1978), new leaves were malformed while older ones showed necrotic spots, the growing point was retarded, and pollen fertility was significantly lowered, when plants were deprived of silicon. Both growth and yield of Si-free plants were substantially less than those of plants grown with Si. Si-free plants, already showing deficiency symptoms, resumed normal growth when Si was resupplied. Similar symptoms were shown after the first stage of flowering for cucumber (Miyake and Takahashi, 1983a), strawberry (Miyake and Takahashi, 1986) and soybean (Miyake and Takahashi, 1985), when they were deprived of silicon. They concluded that silicon was required for normal reproductive growth in these species. Adatia and Besford (1986) found that cucumber plants grown in solution culture at high Si acquired the characteristics of plants grown at high light intensities: shorter petioles, increased fresh weight, dry weight, chlorophyll, RuBP carboxylase activity and soluble protein per unit leaf area, increased rigidity and tougher leaf texture. They suggested that Si additions were beneficial to cucumber growth at all stages.

ii. Interaction between silicon nutrition and other components of higher plant growth

a. Interactions between silicon and other nutrients

The interactions between silicon and various other nutrients have been studied by several researchers. It was initially reported that, under field conditions, additions of silicate increased the yield of grains grown on phosphorus-deficient soils, implying that silicon either enhanced phosphate uptake or could serve as an analog for this element in the plant (Hall and Morrison, 1906). Subsequent research has suggested that, in a soil system, the silicate ion can replace and release the phosphate ion fixed in the soil, thus increasing the amount of phosphate available to the plant, and may also promote the translocation of phosphorus (Takahashi et al., 1990). Under hydroponic conditions, on the other hand, both the content and uptake of phosphorus in rice plants was decreased by the addition of silicon to the culture medium (Ma and Takahashi, 1989). It was shown that silicon-pretreated plants absorbed less phosphorus than those which were grown without silicon, in both +Si and -Si uptake experiments, suggesting that phosphorus uptake may be inhibited by silicon already present in the plant. Calcium, magnesium, iron and manganese levels were found to be lower in plants grown with silicon than in those grown without. No mechanism to account for the effect of silicon on the uptake of these nutrients was proposed. Islam and Saha (1969), reported that addition of silicon to the nutrient solution decreased the uptake of K, Fe, and Mn, while uptake of Ca, P, and Mg increased in rice plants, suggesting that these changes could be accounted for by changes in the ion content of developing silica cells. These cells develop significantly lower K, Fe, Mn and Ca levels but higher Mg levels than surrounding cells as they begin to accumulate high levels of Si (Soni et al., 1972). In studying the effect of silicon additions on P/Zn balance in cucumber plants, Marschner et al. (1990) found that added Si had no effect on P uptake and translocation to the shoot if sufficient Zn were present. However, at low Zn levels, P

supply to the shoot was much higher in the absence of silicon, and Zn supply was depressed, resulting in chlorotic symptoms associated with P-induced Zn deficiency. They suggested that the importance of silicon to plants is in maintaining nutrient balance, perhaps acting as a cation exchanger when deposited in gel form in the apoplasm. The same mechanism is also proposed to account for the alleviation of Mn deficiency symptoms at low Mn (Kluthkouski and Nelson, 1980) as well as Mn toxicity symptoms at high Mn (Marschner, 1986) by the addition of silicon.

b. Effect of silicon on resistance to water stress

Treatment with silicon has been shown to alleviate the symptoms of various disorders in plants. Several species, such as Loblolly Pine (Emadian and Newton, 1989) and rice (Takahashi et al., 1990), have been shown to withstand water stress more successfully if they were grown at high silicon levels. Pine seedlings grown with silicon had a higher growth rate than controls, an effect which was significantly greater if the plants were subjected to conditions of water stress. This enhanced growth was ascribed to higher water and osmotic potentials, greater symplastic water volume, and greater tissue elasticity in the silicon-treated plants. Silicon-treated rice also had a higher growth rate than controls, and again the effect was significantly greater under conditions of water stress. High Si plants under water stress showed less wilting and lower rates of transpiration than low Si plants.

c. Effect of silicon on salinity tolerance

Silicon has also been implicated in tolerance to salinity. Several halophytic species have been shown to accumulate high levels of silicon, and Ahmad et al. (1992) have shown it to play a significant role in salt tolerance in wheat. Dry weight of the shoot increased significantly after silicon addition under saline conditions, and the Na^+ content of both root and shoot was decreased. Germination was also more successful under saline

conditions if silicon was present. It was suggested that silicon may act by sequestering excess sodium in a hydrophilic gel that is then incapable of transfer to the shoot.

d. Effect of silicon on disease resistance

The role of silicon in resistance to pathogens is also one of interest. It was found that silicon rich "halo areas" developed around the point of fungal penetration when plants were inoculated with fungal pathogens. It was postulated that this accumulation of silicon deposits might be induced by fungal metabolites and might constitute a primary defense mechanism against infection (Kunoh and Ishizaki, 1975). Further research into this area by Heath (1981) has given support to the idea that, though it is certainly not the only, and perhaps not the primary mechanism of resistance to fungal attack, silicon deposits at the site of haustorial penetration are an important mechanism of fungal resistance in plants. By studying the interaction of rust fungi with both host and non-host plants, they found that cowpea rust fungus (*Uromyces phaseoli* var. vignae) was unable to form haustoria on French bean (*Phaseolus vulgaris* cv. Pinto) leaves but triggered the formation of electron dense silicon deposits on the mesophyll cell walls closest to the infection hyphae. However, when the leaves were pre-inoculated with extracts from bean leaves infected with French bean rust fungus (*Uromyces phaseoli* var. typica), the silicon deposits did not form and cowpea rust fungus was able to form haustoria and penetrate into the bean leaf cells (Heath, 1981a,b). This led to the conclusion that the ability of the fungus to invade its host species depended on its ability to suppress the development of silicon rich deposits in the mesophyll cell walls of this latter's leaves. In a later paper studying the formation of silicon deposits at haustorial penetration sites in silicon rich and silicon-free bean plants, however, it was found that, though no silicon deposits were formed in silicon-free plants, the penetration of haustoria of cowpea rust fungus was only marginally higher than in silicon rich plants that formed many silicon deposits. This, and information regarding the accumulation of phenolics at the site of haustorial penetration, led to the suggestion that

"either silicon deposition is not the primary barrier to haustorium formation in normal, non-host plants, or a second barrier, such as the impregnation of the plant wall with phenolic materials, comes into play if silicon deposition is prevented" (Stumpf and Heath, 1985). Silicon has been shown to substantially decrease the incidence of blast in rice (Takahashi and Ma, 1990), powdery mildew infection in cucumber (Samuels et al., 1990; Miyake and Takahashi, 1983a; Adatia and Besford, 1986), and various cereals (Takahashi et al., 1990). Silicon is generally thought to be important in resistance to fungal infections, particularly in grasses, but in other plants classified as non-accumulators as well, and is applied under field and glasshouse conditions as an alternative to chemical fungicides.

iii. Silicon Uptake

Uptake of silicon has been examined, in both accumulating and non-accumulating species, by examining the plant absorption of silicon over the entire growth period or over the course of several days. Takahashi et al. (1990) examined silicon accumulation in several species and proposed three modes of silicon uptake in plants: active (in strong accumulators such as rice), passive (in accumulators such as cucumbers) and exclusive (in non-accumulators such as tomato), based on the Si/Ca ratios of these species as outlined above. However, there is considerable disparity in results from various groups. Jones and Handreck studied the accumulation of silicon over 70-102 days in a legume, soybean (1965) and a grass, oats (1969), which, according to the classification mentioned above should have exclusive and active modes of silicon uptake, respectively. In both cases he found that the amount of silicon accumulated by the plants was correlated with the amount of water lost by transpiration, and concluded that in both species silicon uptake was passive. Barber and Shone (1966) also studied silicon uptake by a legume (bean) and a grass (barley) over a period of 4 days. They found that silicon entered both plants at a higher rate than could be accounted for by water movement in the transpirational stream. The two species also contained higher levels of silicon in their xylem exudate than was

present in the external medium. On the basis of these results an active method of silicon accumulation for both species was proposed. Adatia and Besford (1986) studied nutrient uptake in cucumber and tomato plants grown in recirculating nutrient solution over a 7 week period. They observed that, while cucumbers depleted the solution of silicon over time, silicon accumulated in the solutions in which tomatoes were grown. They concluded, therefore, that silicon uptake in cucumbers was active, while in tomatoes, a metabolic exclusion of silicon was likely. Takahashi et al. (1990), on the other hand, found that, over a period of 72 h. during which they measured Si levels in a given amount of solution, cucumbers caused no appreciable change in the Si concentration of the solution, while tomatoes caused the Si concentration in the solution to increase from 0.21 to 0.24 mM, indicating that cucumbers passively absorbed Si while tomatoes actively excluded it. One possible explanation for the differing results of these groups could be the long-term nature of their study periods. If, as Takahashi et al. (1990) have suggested, silicon uptake is related to the developmental stage of the plant, species that exclude silicon during certain developmental stages, while they actively absorb it at others, will perhaps appear to passively absorb the nutrient over their entire growth period. These long term experiments may be useful to classify species regarding their silicon requirements, but may be misleading when used to examine the actual mechanism of uptake. In addition, the effect of treatment conditions on the entire plant, rather than merely the root, can make many long term experiments difficult to interpret. Finally, it would seem that silicon absorption in all species studied, from diatoms to humans, is regulated over a wide range, and is strongly susceptible to conditions of silicon deposition both in the organism and in the environment. Therefore, long term studies may reflect more the properties of a regulatory mechanism than those of the silicon uptake system *per se*.

Van der Vorm (1980) studied the uptake of Si by rice, sugarcane, wheat, sunflower and soybean plants over the course of their growth periods (3-8 weeks) at various concentrations. By comparing the amount of Si actually absorbed by the plant (on

a dry weight basis) to the amount which would have entered the plant passively along with the transpirational stream, he determined that plants are capable of moving from strong accumulation to strong exclusion, depending on species and external Si concentration. For example, while rice and sugarcane accumulated silicon at all concentrations, wheat moved from accumulation of Si at low concentrations to passive absorption of Si at higher concentrations. Soybean, which as a legume would be expected to exclude Si according to the classification scheme of Takahashi et al.(1990), was found to absorb it passively at low concentrations, while sunflower moved from exclusion to accumulation with decreasing concentration of Si. This study underlines the variability of Si uptake, especially in species which are not strong accumulators.

III. SILICON IN RICE

Given the growing body of information pointing to silicon as beneficial to plant growth, if not as an important plant nutrient, it is essential at this time to examine possible physiological roles for the element as well as the mechanisms by which it is absorbed by plant roots. I propose to address the latter of these questions. As the agronomic importance of silicon in rice culture is already well established, and rice has been shown to be one of the strongest silicon accumulators in the plant kingdom (Takahashi et al. 1990), it is the obvious choice as a primary model system in which to study this process. Cucumbers, in which the role of silicon was discussed in previous sections, will also be considered in the present study to provide a comparison to silicon absorption in rice plants.

A. EFFECT OF SILICON ON RICE GROWTH

Rice has been shown to accumulate up to 10% (on a dry weight basis) Si under field conditions, whereas other grasses contain only 1 to 2% and dicots typically contain only 0.002 to 0.02%. Insoluble silicon (as silica gel or polysilicic acid) is the most prevalent form, comprising 90% of all silicon in the rice plant, while monosilicic acid

accounts for 0.5 to 8% and colloidal silicon accounts for 0 to 3.3% of silicon present (Yoshida, 1962a). The primary site of silicon deposition in rice is in the cell wall. Roots of the rice plant contain the least silicon deposits, and these reveal no pattern of deposition, being widely distributed in all root tissue and consisting of a silicification of the cell walls. The endodermis shows especially strong silicification but only of the inner tangential and radial walls. Most of the monosilicic acid absorbed by the rice plant is transported to, and deposited in, the shoot, in which the cell walls of all tissues are silicified to some extent (Parry et.al., 1984). Silicon content is particularly high in older tissues and those with high rates of transpiration, as well as in stelar elements, along with bundle sheath and schlerenchyma cells, and epidermal cells. Lowest levels of silicon deposition are in meristematic tissues and specialized tissues such as the geotropically sensitive pulvinus (Kaufmann et al., 1981). Specialized epidermal cells, such as trichomes and cork silica cell (CSC) pairs contain especially high silicon levels. The silica cell of the CSC pair loses its cytoplasm as it develops and its lumen fills completely with silica gel, while the cork cell retains its cytoplasm and develops significantly higher levels of mitochondria and endoplasmic reticulum. In addition, the pair develops highly thickened and silicified walls (Kaufmann et al., 1981). These cell pairs are thought to provide rigidity to the plant, acting somewhat like a skeleton for the leaves. Yoshida et al. (1962c) also found that silicon deposits were particularly heavy at the level of the cuticle. Alternate layers of silicon and cutin are deposited at the surface of the epidermal cell, providing further rigidity to the leaf.

The structural role silicon plays in rice, by virtue of cell wall deposits, CSC pairs, and epidermal deposits, has generally been considered its most significant functional role. Silicon applications prevent rice from lodging, promote a more upright growth habit, which may promote photosynthesis and gas exchange in the community, and prevent excessively drooping and tender leaf growth, especially under conditions of heavy ammonium fertilization (Takahashi et al., 1990). This is ascribed to the added rigidity of

the plant due to silicified walls and specialized cells. Silicon also prevents excessive water loss, possibly due to the silica-cuticle double layer, which prevents water loss through the epidermis This layer is also thought to protect against fungal and insect attack. Certain chewing insects have been found to have their mandibles damaged by the double layer and be unable to penetrate it, and fungal infection is considerably reduced in silicon-treated rice populations (Yoshida et al. 1962c). Okuda and Takahashi (1961c), however, by studying rice growth under conditions free from lodging, mutual shading or other conditions which silicon would alleviate due to mechanical effects, determined that silicon must also play a strictly physiological role, as Si-free plants still had lower grain yield, fresh and dry weight, and top length than did +Si plants. They observed much higher rates of water loss from the leaves of -Si plants, as well as increased rates of P absorption, and remarkably increased rates of Mn and Fe uptake. However, translocation of ^{32}P to meristematic areas was increased at high Si levels, even though uptake of the isotope was lower than in low Si plants. They determined that plants were particularly susceptible to Si deprivation at the reproductive stage of growth (Okuda and Takahashi, 1961b). They concluded that silicon must play an important physiological role in addition to a purely mechanical one. This physiological role has yet to be determined.

B. UPTAKE OF SILICON BY RICE PLANTS

Okuda and Takahashi (1962c) examined the physiological basis of silicon absorption by plants. They found that, over 37 h., rice significantly reduced the level of silicon in the nutrient solution while tomatoes did not. The concentration of silicon in the xylem exudate of rice plants was found to be several times higher than it was in the external medium after this period. They concluded that rice plants were able to selectively accumulate silicon. As this selectivity was lost if the roots were removed from the plant, they concluded that the specificity of silicon uptake was due to a mechanism in the plant roots. Germanium uptake in rice was measured in the presence and absence of silicon in an

attempt to determine whether a carrier specific for silicon existed (Takahashi et al., 1976).

It was found that germanium uptake was substantially reduced in the presence of silicon.

As germanium is considered an analog of silicon (Azam et al., 1974), it was concluded

that the two compounds were being absorbed by a carrier specific for silicon in the roots,

which absorbed silicon in preference to germanium. The effect of metabolic inhibitors on

uptake was studied to determine whether silicon uptake was a metabolically dependent

process (Okuda and Takahashi, 1962b). Silicon and phosphorus levels were measured in

the nutrient solution containing various inhibitors of respiration 24 h after introduction of

the plants. Silicon uptake was found to be as strongly inhibited as was phosphorus uptake.

They concluded that silicon uptake was strongly dependent on metabolic energy. Mitsui

and Takatoh (1962), using ^{31}Si as a tracer, found that similar treatment with inhibitors

significantly decreased transpiration as well as silicon uptake. They also found that, unlike

^{32}P, which remained mostly in the root, most of the ^{31}Si was rapidly translocated to the

shoot. They concluded that a substantial portion of silicon uptake in rice was

transpiration-dependent, and that the inhibitors acted on silicon uptake by reducing

transpiration. The conflicting results of these two groups may be due to the duration of

exposure of the plants to inhibitors in both cases, causing all plant processes to be

affected, rather than merely those of the root, introducing the possibility of substantial

error in interpreting the results. Takahashi and Hino (1978) studied the effect of pH on

silicon uptake and found that silicon uptake was decreased at pH 11, at which point silicon

would be in the form of the silicate anion, which led them to conclude that a silicon

transport mechanism exists which is specific for the uncharged form of silicon. However,

it is highly likely that the membranes of root cells could be adversely affected by such an

elevated pH. It is noteworthy that the pH optimum for silicon uptake in marine diatoms

has been reported as pH 9.5, implying an involvement of the silicate anion in transport

(Sullivan 1986).

These studies leave unresolved a number of questions regarding the uptake of silicon by rice roots. In the first place, long term studies, utilized in most examinations of silicon uptake, are more representative of total silicon metabolism and deposition patterns in the plant than of silicon uptake by the roots. Therefore, the present study utilizes the shortest study periods possible so that the results more accurately represent the process involved in silicon absorption by the roots. Furthermore, this study will attempt to utilize approaches that have been successfully applied in more fully studied systems (i.e. diatoms and mammals), such as kinetic analyses, short term exposure to inhibitors and non-invasive treatments for the modification of metabolic functions. With the resolution of certain basic questions regarding the nature of the silicon uptake system in rice, it is hoped that further studies will allow a full characterization of this uptake system.

MATERIALS AND METHODS

I.GROWTH CONDITIONS

A. RICE

i. Seeds

Rice (*Oryza sativa* L. cv. M102) seeds were obtained from J.E. Hill of the California Cooperative Extension, Davis, Ca.

Prior to planting, seeds were soaked for 45 minutes in a 1% solution of hypochlorite. They were then rinsed with distilled water and soaked with aeration for 24 h. Soaked seeds were planted on mesh discs and germinated over water, in the dark at 30°C, for three days before being transferred to hydroponic tanks.

ii. Growth medium formulation

The medium used for the growth of plants and as the uptake solution is a modification of Johnson's solution (Table 1) corresponding to approximately a 1/5 dilute Johnson's solution. For plant growth, 0.5 mM Si was added to the solution as Na - metasilicate unless otherwise stated. The pH of the solution was maintained at 6.0 by daily monitoring and additions of $CaCO_3$. Supply solution to the tanks was identical to the growth solution but at twice the nutrient concentrations.

iii. Plant growth

Plants were grown in a recirculating hydroponic system which was topped up continuously with fresh nutrient solution, delivered by a peristaltic pump at approximately 100 ml h^{-1}, in order to maintain a constant level of nutrients in the growth solution. This was monitored by measuring the nitrate and potassium levels in the tank on a daily basis and adjusting the flow of the supply solution accordingly. The tanks used were of black Plexiglas to prevent light from reaching the roots. The solution was continuously aerated by bubbling air through an airstone below the surface of the solution in a separate

Table 1 : Rice growth medium formulation

COMPOUND	CONCENTRATION (μM)
Macronutrients:	
KNO_3	800
$Ca(NO_3)_2 . 4H_2O$	800
NH_4NO_3	400
KH_2PO_4	400
$MgSO_4 . 7H_2O$	200
Micronutrients:	
KCl	10.0
$MnSO_4 . H_2O$	0.4
H_3BO_4	5.0
$ZnSO_4 . 7H_2O$	0.4
$CuSO_4 . 5H_2O$	0.1
H_2MoO_4	0.1
Fe (as Na-Fe-EDTA)	4.0

aeration/mixing section of the growth tank. Solution was pumped from the aeration/mixing section into the growing compartment by means of a Brinkman circulating pump (Model T1). Environmental conditions were maintained at 25°C, 60 % R.H and a photoperiod consisting of 16 hours of light and 8 hours of darkness. Light was provided by Vitalite (Durotest 96T12, USA) full spectrum lights, and was kept at an irradiance of approximately 500 $\mu E.m^{-2}.s^{-1}$ at the leaf surface.

B. CUCUMBERS

i. Seeds

Cucumber (*Cucumis sativus*)seeds were obtained from De Reuter Seeds, St. Catherines, Ontario. The variety used for all experiments was Corona Long English.

Prior to planting, seeds were soaked for 10 minutes in a 1% solution of hypochlorite. They were then rinsed with distilled water and laid on wet filter paper in a sealed petri dish to germinate. They were germinated in the dark for 4 days and then each seedling was transferred to hydroponic tanks held singly in foam plugs in collared discs.

ii. Growth medium formulation

The medium used for both the growth of plants and as the uptake solution was a modification of the solution used by commercial greenhouse growers to raise cucumbers (Table 2). For plant growth, 0.5 mM Si was added to the solution as Na - metasilicate, or as Kasil 6, a viscous potassium silicate solution (4.1 mmol $SiO_2 . ml^{-1}$). The pH of the solution was maintained at pH 6 by daily monitoring and additions of $CaCO_3$. Supply solution to the tanks was identical to the growth solution but at twice the concentrations of nutrients.

iii. Plant growth

Growth conditions were the same as those described for rice.

Table 2 : Cucumber Growth medium formulation

COMPOUND	CONCENTRATION (μM)
Macronutrients:	
KNO_3	1100
$Ca(NO_3)_2 . 4H_2O$	750
KH_2PO_4	300
$MgSO_4 . 7H_2O$	200
Micronutrients	
KCl	10.0
$MnSO_4 . H_2O$	1.1
H_3BO_4	9.2
$ZnSO_4 . 7H_2O$	0.3
$CuSO_4 . 5H_2O$	0.1
H_2MoO_4	0.1
Fe (as Na-Fe-EDTA)	4.0

C. TREATMENT OF EQUIPMENT

To avoid contamination of experimental and growth solution with exogenous silicon, only plastic or Plexiglas vessels were used to handle the solutions. All water used for experimental solutions was distilled and deionized. Water for growth solutions was distilled. It was determined that glass distilled water was not significantly contaminated with silicon, as it showed no color development when assayed for Si (see methodology below).

II. ASSAY METHODS

A. SILICON

The amount of silicon present in both uptake and nutrient solution was determined absorptiometrically. The basic method was that described by Strickland and Parsons (1968), with some modifications as described below. All solutions were stored in polyethylene bottles, and reactions were carried out in plastic tubes, as the use of borosilicate glass tubes or bottles at any stage in the procedure produced higher blank values. This finding is in accordance with that of Fanning and Pilson (1973). The assays were conducted on a scale approximately 40 times smaller than the published methods, which resulted in a higher variability than the large scale assay. It was found that this variability was minimized if the time of reaction between the molybdate reagent and the sample was reduced to 10 minutes and if no less than 30 seconds difference in incubation time of the unreduced β-molybdosilicic acid existed between the samples and the standards.

The method is based on the absorptiometric measurement of reduced β-molybdosilicic acid at 810 nm. Some of the problems associated with the measurement of silicon in the β form of the acid include considerable interference from salts present in the nutrient solution, conversion to the α form, and decay of the reduced compound under

conditions which dissolve molybdophosphoric acid (Morrison and Wilson 1963a). To minimize these problems, several modifications were made to the initial method. Samples were diluted 10 to 20 times with distilled water to minimize interference from salts. As the formation of β-molybdosilicic acid over α-molybdosilicic acid is favored at lower pH (Morrison and Wilson 1963a), the molybdate reagent was further acidified to bring the final reaction pH to 3.0. This reduced variability between replicates and increased the linearity of the standard curve. In addition, it was found that no reactive silica remained in the sample if this latter was frozen prior to analysis. Therefore, all samples were stored at 4 °C.

Under experimental conditions where rapid analysis of many samples was required, a method was developed by which large batches of samples could be analyzed simultaneously, while preserving the maximum 30 second difference in incubation time of the unreduced β-molybdosilicic between first and last sample. Samples were analyzed in 96 well microtiter plates, and the volume of the reaction was reduced a further 10 times. As all the samples are read within 22-25 seconds of one another by the plate reader, the absorption of unreduced β-monosilicic acid at 410 nm can be read (phosphoric acid complexes are dissolved by tartaric acid added immediately before measurement -- Morrison and Wilson, 1963b). This not only allows up to 80 samples to be read simultaneously, but also reduces the total time of analysis by one hour.

B. POTASSIUM

The amount of potassium present in growth and uptake solutions was measured by flame photometry. The flame photometer used was an Instrumentation Laboratory 443, calibrated using two samples of K_2SO_4 of known concentration. Samples were diluted tenfold and added to 9 ml of $LiNO_3$ prior to measurement.

C. NITRATE

Nitrate was measured colorimetrically by the method described by Cataldo et al.(1975)

III. UPTAKE CONDITIONS

A. GENERAL

The environmental conditions for uptake were the same as those for the growth of the plants unless otherwise stated. Uptake experiments were conducted in black Plexiglas vessels, holding 750 ml of solution for experiments using rice and 3L of solution for experiments using cucumbers. In uptake solution, silicon was added as monosilicic acid, made by passing a solution of Na - metasilicate through a cation exchange resin (Dowex 50W (50x8-200)) in acid (H^+) form, or as Na - metasilicate. Samples of the ambient solution were taken at regular intervals and analyzed immediately for silicon and, for selected experiments, potassium. The uptake of Si and K was measured by depletion. Plants were harvested immediately following the experiment and the fresh weight of root and shoot recorded. Uptake is expressed in $\mu mol.g\ root^{-1}.h^{-1}$.

B. VOLUME MAINTENANCE

During all uptake experiments it was necessary to precisely monitor and maintain solution volume, to determine the volume of solution lost to transpiration and to guard against concentration effects should the transpiration rate be higher than the rate of silicon uptake; two methods were used. In the first, vessels were weighed at each sample time and their weight was restored to its initial level by the addition of silicon-free growth medium. In the second, a capillary tube was attached to the side of the uptake vessel (Figure 1) and calibrated to allow precise measurement of the volume of solution in the vessel. The solution was returned to the starting level, using silicon-free uptake medium, before each sample was taken for analysis.

FIGURE 1: UPTAKE VESSELS WITH RICE PLANTS

C. CONCENTRATION DEPENDENCE

i. Cucumbers

Eight experimental treatments were used, consisting of eight uptake solutions of differing silicon concentrations ([Si]): 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 mM Si. All other conditions were the same as the growth conditions previously described. Samples were collected every 30 minutes for a period of 4 hours and analyzed for silicon.

ii. Rice

Twelve experimental treatments were used, consisting of twelve uptake solutions of differing silicon concentrations ([Si]): 0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, 1.0, 1.5, 2.0, 3.0 mM Si. All other conditions were the same as the growth conditions previously described. Samples were collected every 15 minutes for a period of 105 minutes and analyzed for silicon.

D. EFFECT OF SILICON PRETREATMENT

i. Cucumber

a. Pretreatments

Plants were grown in 0, 0.5, and 1.0 mM Si for one week before the experiment. All other growth conditions were maintained as previously described.

b. Uptake Conditions

The experimental solution contained 1 mM Si for all 3 treatments. All other conditions were the same as the growth conditions. Samples were taken every 4 h for a period of 32 h. The samples were analyzed immediately for silicon.

ii. Rice

a. Pretreatments

Plants were grown for three weeks in either 0 or 1 mM silicon. Si levels were monitored in both treatment solutions and maintained by the addition of fresh solution daily. All other growth conditions were maintained as previously described.

b. Uptake Conditions

Three concentrations of Si were applied: 0.5, 1.0, and 2.0 mM Si. All other conditions were the same as the growth conditions. Samples were taken every 40 minutes over a period of 4 h. The samples were analyzed immediately for silicon.

E. TRANSPIRATION DEPENDENCE OF SILICON UPTAKE (RICE)

Uptake was measured at differing rates of transpiration from plants grown as previously described. Environmental conditions were as previously described for plant growth with the following exceptions: temperature was held between 20° and 25°C; irradiance was approximately 400 $\mu E.m^{-2}.s^{-1}$ and ambient R.H. was approximately 40%. A low transpiration treatment was achieved by covering the plants with a tent of clear plastic. A moderate transpiration treatment was obtained by covering the plants with a tent made of clear plastic into which holes had been cut. The plants in the high transpiration treatment were left uncovered. All other conditions were the same as the growth conditions previously described. Initial Si concentration during the measurement of Si uptake was 0.5 mM Si. Samples were taken every 40 minutes for 200 minutes and analyzed immediately for silicon. Volumes of solution lost to transpiration were also recorded at each sampling time.

F. TEMPERATURE DEPENDENCE OF SILICON UPTAKE (RICE)

Uptake was measured under 5 different temperatures: 10, 15, 20, 25, and 30 °C. All other conditions were the same as the growth conditions previously described. Samples were collected every 30 minutes for 3 hours and immediately analyzed for potassium and silicon levels. Volumes of solution lost to transpiration were also recorded at each sampling time.

G. EFFECT OF ANOXIA ON SILICON UPTAKE (RICE)

Uptake of Si was measured from a 0.5 mM Si solution in growth medium of the same chemical composition as was used for growth of the plants. Control plants were placed in solution through which air was bubbled, as it was during plant growth. The remaining plants were placed in solution through which nitrogen gas was bubbled to drive off the oxygen and subject the roots to anoxic conditions. Before the start of the experiment, the anoxic solutions were treated with nitrogen bubbling for thirty minutes to insure the complete removal of oxygen from the solution. Samples were collected every 30 minutes for a period of 3 hours, and immediately analyzed for silicon and potassium. All other conditions were the same as the growth conditions previously described with the following exceptions: temperature was held between 20° and 25°C, irradiance was approximately 400 $\mu E.m^{-2}.s^{-1}$ and ambient R.H was approximately 40%. Volumes of solution lost to transpiration were also recorded at each sampling time.

H. CYANIDE INHIBITION (RICE)

Uptake of Si was measured from a 0.5 mM Si solution in growth medium identical to that used for growth of the plants. Half of the plants were exposed to 1 mM KCN (treatment) 15 minutes prior to the start of the Si measurement period and throughout the experiment. The uptake solution without KCN (control) was supplemented with K_2SO_4 to compensate for the addition of extra potassium with the cyanide in the treatment solution. Environmental conditions were as previously described for plant growth with the

following exceptions: temperature was held between 20° and 25°C, irradiance was approximately 400 $\mu E.m^{-2}.s^{-1}$ and ambient R.H was approximately 40%. Samples were collected every 15 minutes for a period of 1 hour. They were analyzed immediately for silicon. Potassium analyses were also performed so that the uptake of potassium could be used as an internal control. As uptake of potassium has been well characterized in other studies, monitoring of potassium uptake under present experimental conditions will serve as a standard of comparison for assessment of the validity of results obtained under these conditions for Si uptake.

RESULTS AND DISCUSSION

I. SILICON UPTAKE BY RICE

A. TIME DEPENDENCE

Two experiments were done to examine the time dependency of silicon uptake (Figure 2). In both experiments the plants completely depleted the solution in 18 h. Initial uptake rate in both cases was 8.7 μmol.g root^{-1}.h^{-1}, and this rate was relatively stable for the first six hours, but then decreased, due most likely to the decreasing Si levels in the solution. The average rates over the experimental period were 5.2 and 6.4 μmol.g root^{-1}.h^{-1}.

Figure 2: Depletion of [Si] by Rice as a Function of Time

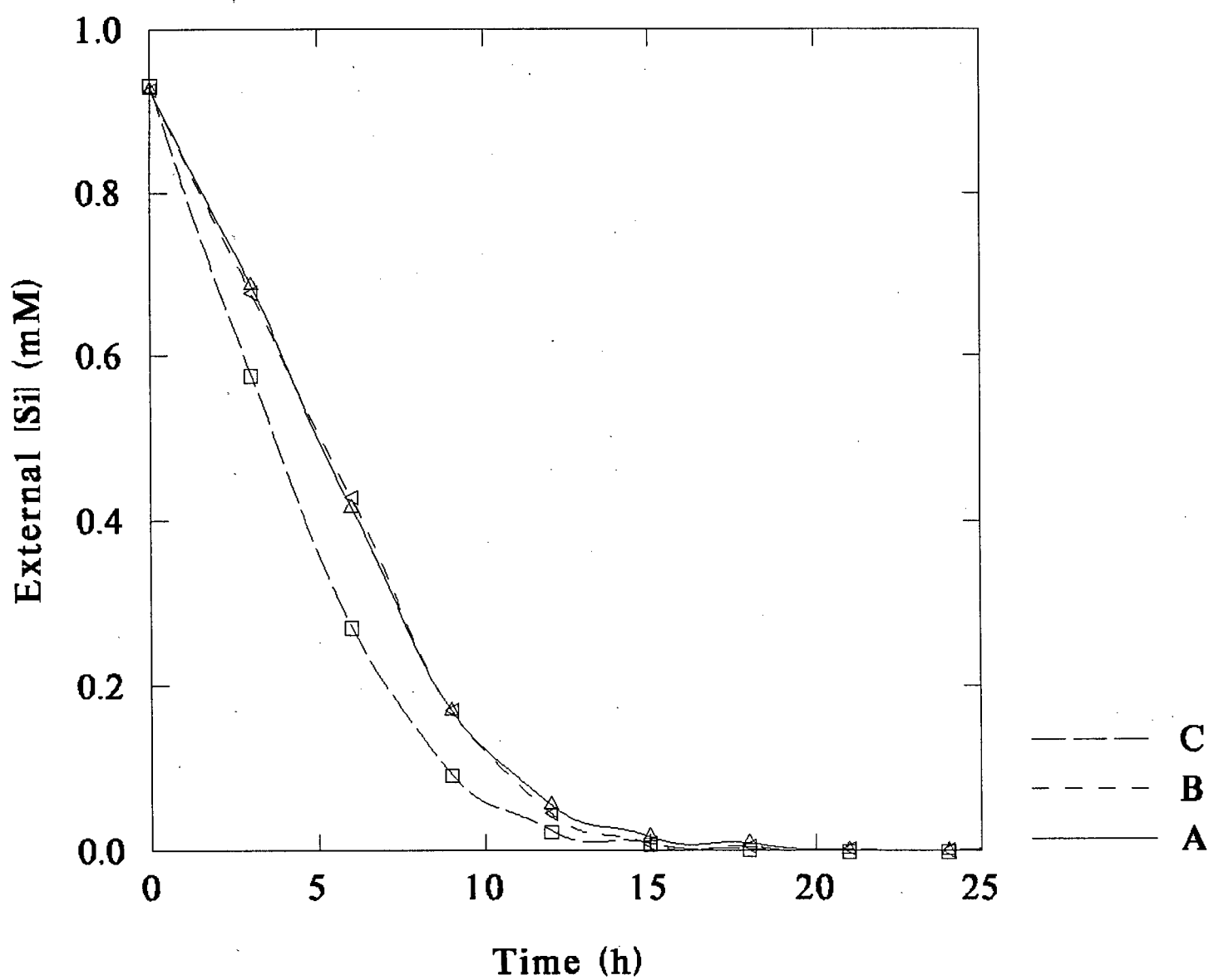Temp = 25°C, RH = 60%, LI = 500 μE.m^{-2}.s^{-1}

Initial [Si] = 1 mM

3 replicates represented by lines A(———), B(----), and C(---)

Samples every h for 30 h.

Figure 2: Uptake of Si by
Rice Roots over Time

B. CONCENTRATION DEPENDENCE

The plot of rate of uptake as a function of concentration may be described by Michaelis-Menten kinetics, indicating a saturable mechanism responsible for the absorption of silicon (Figure 3). The system appears to saturate at approximately 2 mM external silicon. Analysis of the data by a Hofstee plot (Figure 4) shows that the system had a V_{max} of 18.5 μmol.g root^{-1}.h^{-1} and a K_m of 0.587 mM. This K_m value is higher by a factor of 10 than the reported K_m values of most other nutrient transport systems (Epstein, 1972 -- Table 6.1), indicating that Si uptake has a much lower affinity system than most transport systems studied. However, the average concentration of available Si in the soil environment is approximately 0.5 mM (Birchall, 1978). An uptake system with a K_m in this range (0.5 mM) is to be expected, as it would provide the plant with the maximum flexibility with regard to the maintenance of adequate Si levels in varying soil [Si] conditions.

The only other group of organisms for which a kinetic analysis of Si uptake has been undertaken is the diatoms. Diatom systems studied were found to have much lower K_m values than found here for rice, ranging from 1.4 to 4.5 μM in unstarved cultures (Azam et al., 1974; Nelson et al., 1976). As the average concentration of available Si in sea water is much lower than in the soil solution (0.00-0.05 mM) (Birchall, 1978), one would expect a higher affinity system in these organisms than in higher plants. The diatoms were also found to have two transport systems: a high affinity, low capacity system and a low affinity high capacity system (Azam et al., 1974). In the present study, there was no indication of more than one transport system, but it is possible that one may exist at external Si concentrations higher than 3 mM, the highest concentration used here. This is, however, somewhat unlikely given that solutions greater than 5 mM $Si(OH)_4$ polymerize to form silica gel or colloidal silica (Alexander et al., 1954), and that such solutions would be highly unlikely to occur in the external environment.

Figure 3: Concentration Dependence of Si Uptake by Rice

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial external [Si] = 0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, 1.0, 1.5, 20., 3.0 mM

Samples every 15 min for 105 min.

Error bars represent 95% confidence limits.

Figure 3: Concentration Dependence
of Si Uptake by Rice
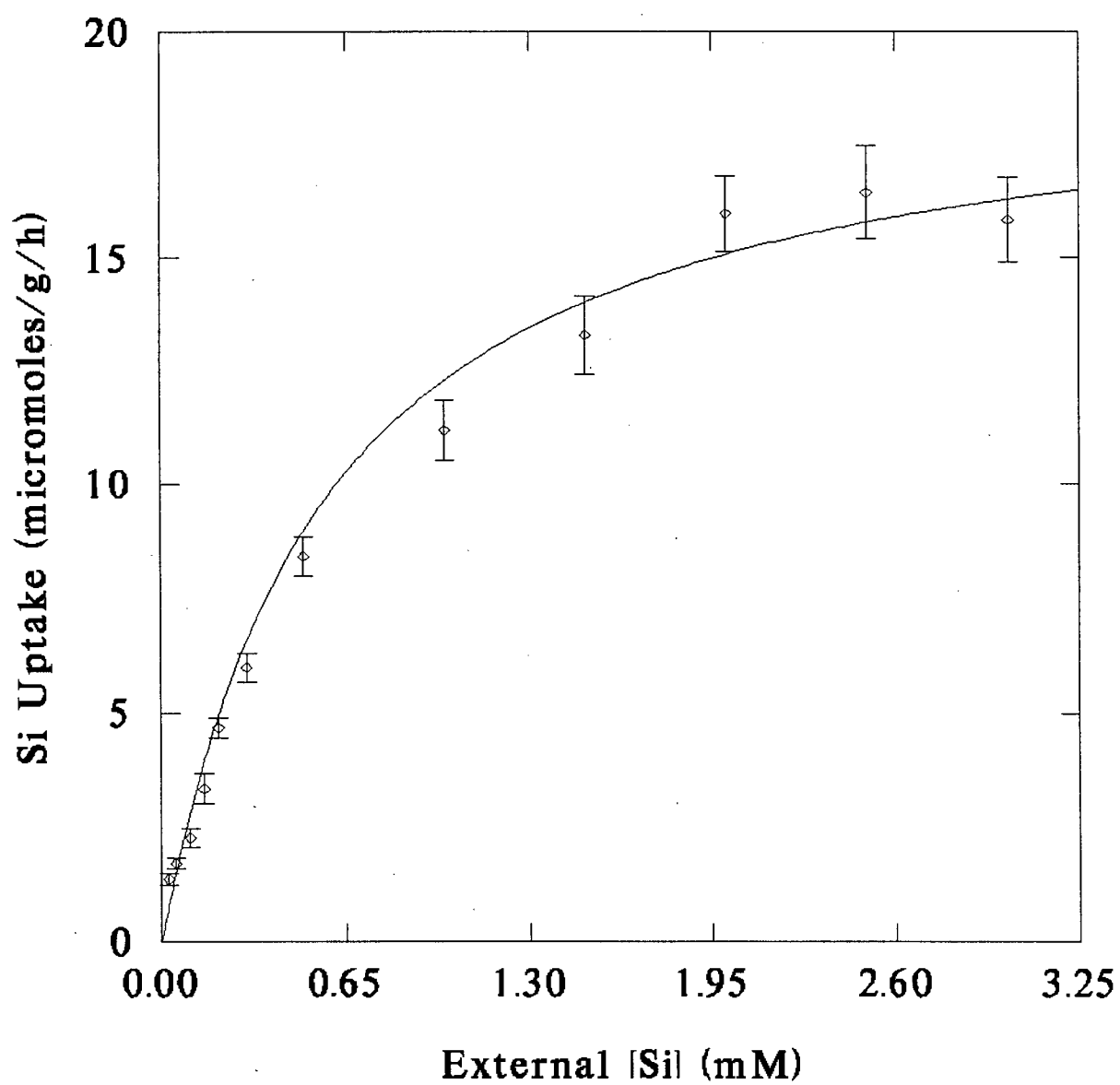
Figure 4: Hofstee plot of Si Uptake by Rice

Data used are those represented in Figure 3.

V represents uptake (μmol.g^{-1}.h^{-1}). [S] represents substrate concentration (mM).

Figure 4: Hofstee Plot of
Si Uptake by Rice
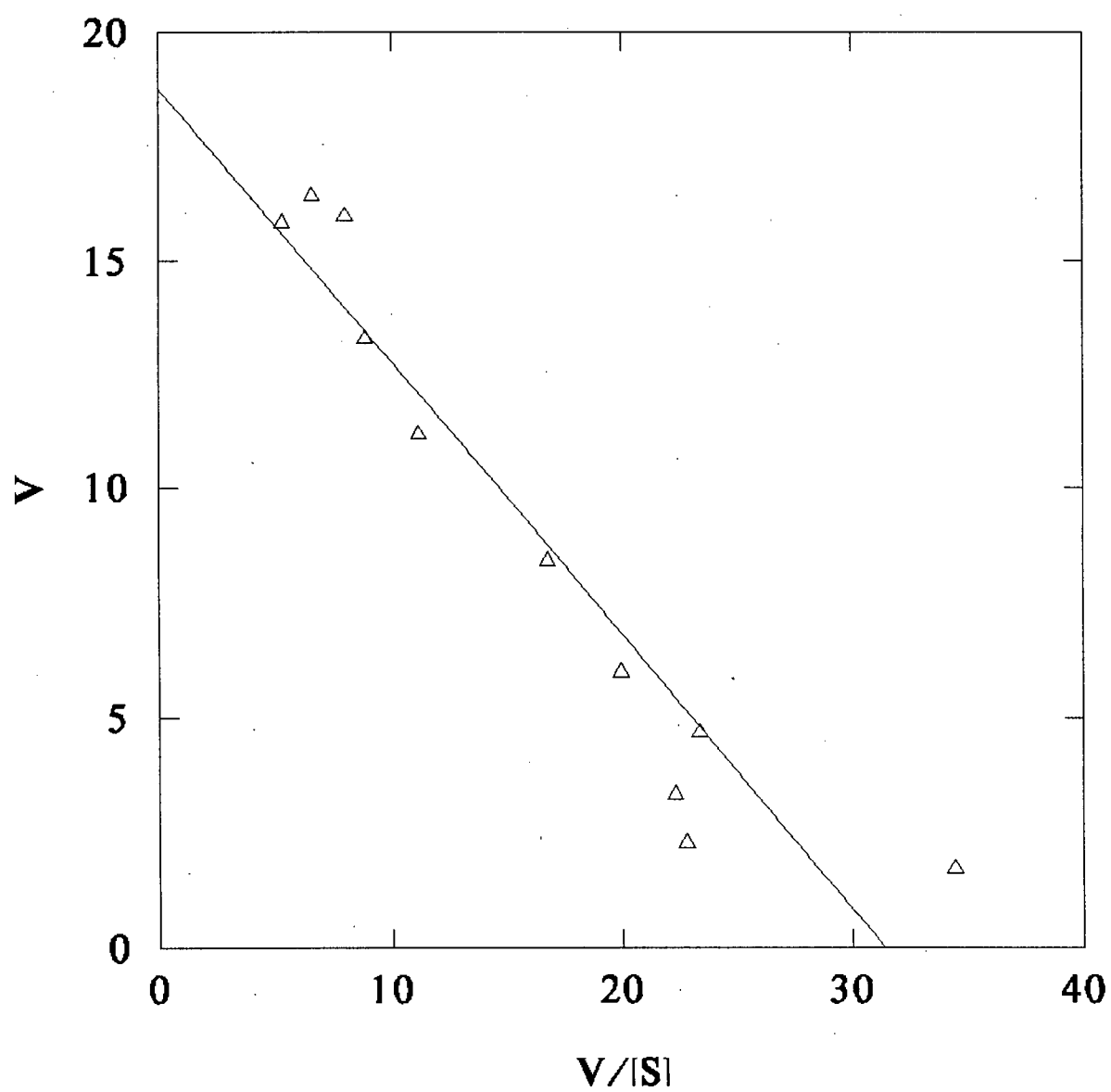
C. EFFECT OF VARIABLE TRANSPIRATION RATES

Evidence of a saturable mechanism for Si absorption would make it seem unlikely that Si transport was occurring passively along the transpiration stream, as has been previously suggested (Jones and Handreck, 1965; Mitsui and Takatoh, 1962; Hutton and Norrish, 1974; Bennett, 1982). The data presented in Figure 5 would seem to reinforce the idea of silicon uptake as a process distinct from transpiration. There was no correlation between rates of transpiration and rates of Si uptake. As the level of relative humidity increased, the rate of transpiration decreased. The rate of silicon uptake, however, did not decrease with decreasing transpiration, and was even seen to increase slightly at higher relative humidity, though the difference was minimal. This further supports the conclusion that silicon uptake is not occurring along the transpiration stream but is occurring by some other, presumably metabolically-dependent, process.

Further support is given to this view by the comparison of the actual amount of silicon taken up by the plants (Total Uptake) with the amount of uptake that would be expected, had this process occurred merely by passive movement of solution via the transpiration stream (Expected Uptake: Figure 6). Total uptake represents the amount of silicon actually absorbed. Expected uptake is calculated by multiplying the volume of water transpired by the plant by the concentration of silicon in the external solution. Evidently, as the actual level of uptake was 8 to 12 times higher than that which it would be possible to obtain by transpiration-driven means alone, silicon is not absorbed passively through the transpiration stream.

The results in Figure 7 also illustrate the independence of uptake from transpiration. In general, transpiration increased slowly and linearly as the ambient temperature increased. Uptake, however, increased by much larger increments, reaching a maximum rate of 9.292 μmol.g root^{-1}.h^{-1} at 25°C and decreasing again as temperature continued to increase. This would indicate that uptake cannot be occurring passively along

the transpiration stream, as there was no similarity between the temperature dependence of uptake and transpiration.

<u>Figure 5: Effect of Variable Transpiration Rates on Uptake</u>

<u>of Si by Rice Plants</u>

Temp = 25°C, LI ~ 500 $\mu E.m^{-2}.s^{-1}$

Low RH=50%, Medium RH~70-80%, High RH=100%

Initial [Si] = 0.5 mM Si

Samples every 40 min for 200 min.

Error bars represent 95% confidence limits.

Figure 5: Effect of Variable Transpiration Rates on Uptake of Silicon by Rice Plants
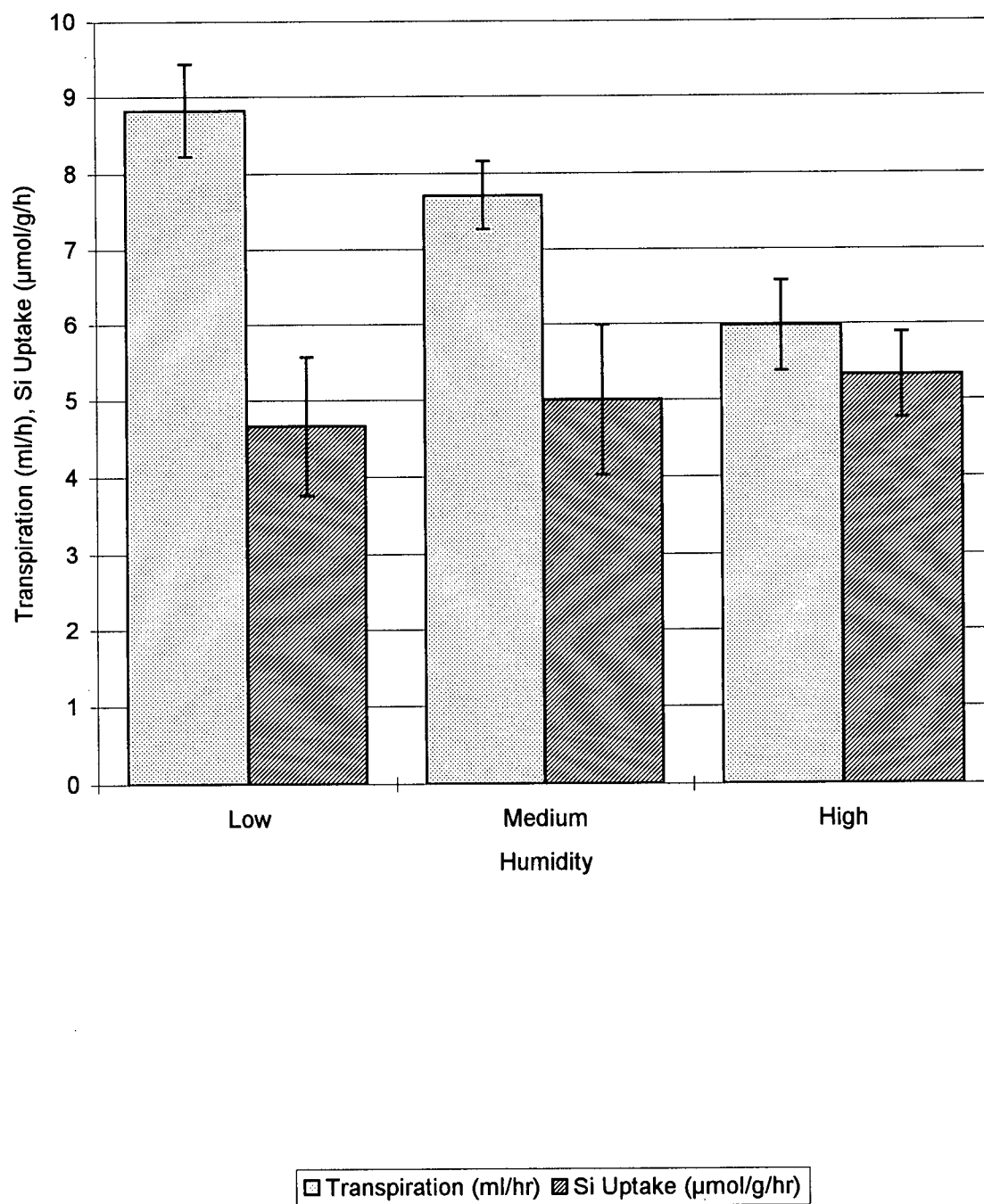
<u>Figure 6: Expected Transpiration-Driven Uptake Based on</u>

<u>Passive Entry of Si by Rice Plants Compared to Actual</u>

<u>Uptake</u>

Expected Uptake = Volume lost to transpiration $\times$ External [Si]

Actual uptake reflects data from Figure 5.

Figure 6: Expected Transpiration-Driven Uptake Based on Passive Entry of Silicon by Rice Plants as Compared to Actual Uptake
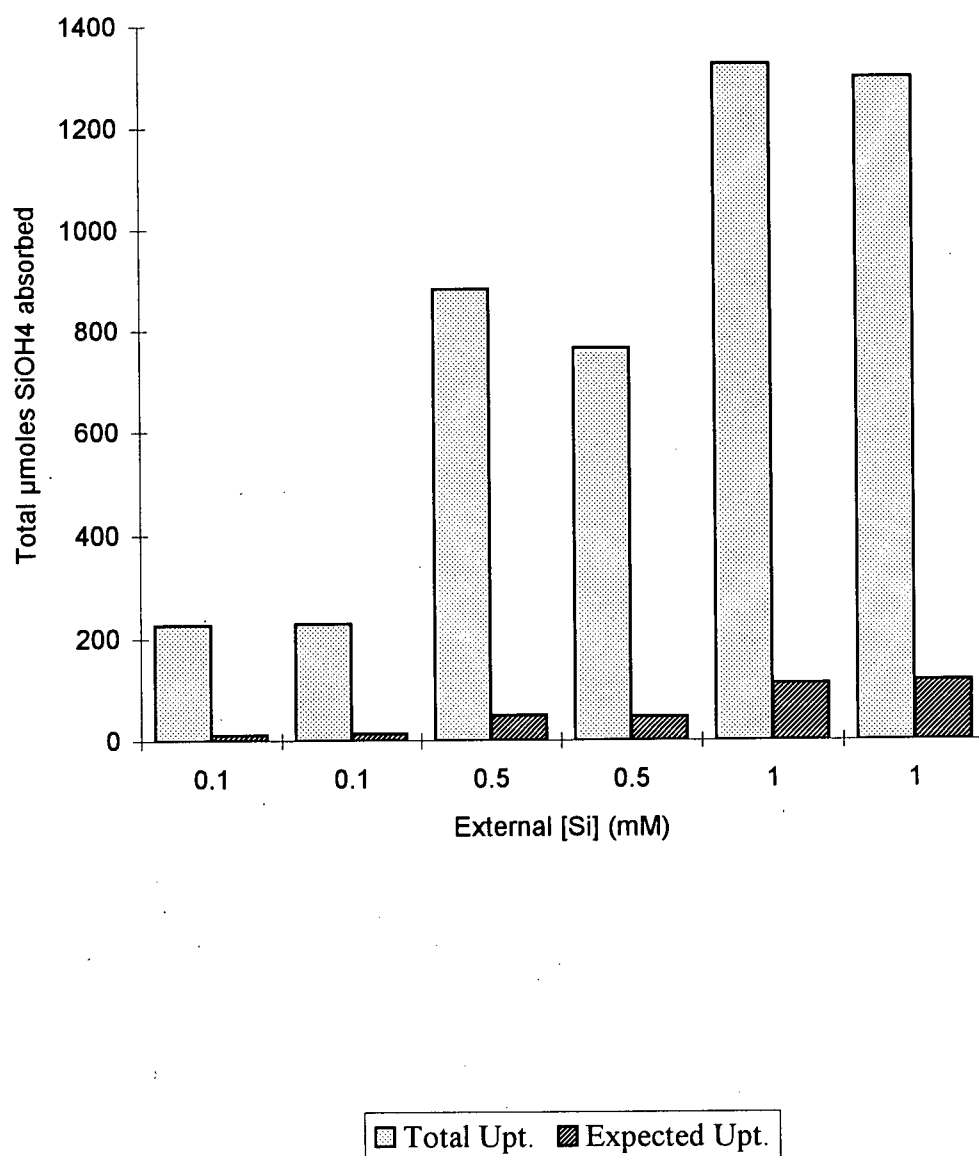
D. EFFECT OF TEMPERATURE

The effect of varying temperatures on uptake has been used in the past to confirm the metabolic dependence of various ion transport systems (Glass et al., 1990a; Glass, 1988; Glass and Siddiqi, 1982; Clarkson and Warner, 1979; Epstein et al., 1962). Within the range of approximately 10 to 30°C an increase of 10°C usually causes the rate of metabolic processes to increase by a factor of 2 or more, hence Q_{10} values of 2 or higher are characteristic of metabolic processes. Purely physical processes such as ion exchange are much less temperature sensitive and generally have temperature coefficients (Q_{10}) close to 1 (Epstein, 1972). Q_{10} values for the data shown in Figure 7 are listed in Table 3. The Q_{10} values were calculated as $(U_2/U_1)^{10/t_2-t_1}$, where U_1 and U_2 are the uptake rates at temperatures t_1 and t_2 , respectively. The Q_{10} values for transpiration varied from 1.0 to 1.6, with an average value of 1.3. This value indicates a lack of dependence on metabolic energy. The Q_{10} for Si uptake between 10 and 15°C was also near one, which would indicate a passive flux. However, as rice is very sensitive to low temperatures, this may have been due to chilling damage to the plant. Above 15°C the Q_{10} values for Si uptake were all above 2, and they ranged overall from 1.2 to 5.2, with an average value of 3.6. Such a high value indicates a metabolically-dependent uptake process. Potassium uptake was measured to serve as an internal control (Table 3), and the calculated Q_{10} values were similar to those previously reported for that element (Glass and Siddiqi, 1982; Epstein et al., 1962). An earlier study of K^+ uptake in rice (Erdei and Zsoldos, 1977) reported Q_{10} values almost identical to those found in this case. Silicon uptake displayed a dependence on temperature of similar magnitude to that for potassium uptake by the high affinity potassium transport system, as well as that for the high affinity uptake systems for other nutrients such as nitrate (Glass et al., 1990b), ammonium (Wang et al., 1993a) and sucrose (Saftner et al., 1983). Si may therefore be transported by an active uptake process similar

to those postulated for these elements. This reinforces the conclusion that silicon absorption in rice relies on an energy-dependent transport process.

Silicon transport systems in diatoms have also shown Q_{10} values of ~2 as well as Michaelis-Menten type saturation kinetics (Azam et al., 1974), and have been shown to be dependent on the Na^+ gradient established by the Na^+/K^+ ATPase. It was further demonstrated that Si is symported with Na^+ by one or both of two silicon specific molecules (Battacharyya and Volcani, 1980;1984). It would further our understanding of silicon transport in higher plants if it could be determined whether or not Si transport is dependent on the H^+ gradient across the plasmalemma, as well as whether or not the silicon specific ionophores thought to be responsible for Si transport in diatoms (Sullivan, 1986) have any similarities with molecules found in higher plants.

Table 3: Q_{10} Values for Uptake and Transpiration at Various Temperatures for Plants Grown at 25°C

Temp.	Q_{10}		
	Si Uptake	K Uptake	Transpiration
10-15°C	1.2	3.6	1.6
15-20°C	5.2	2.6	1.2
20-25°C	2.7	2.7	1.1
25-30°C	3.0	2.8	1.4

Figure 7: Temperature Dependence of Si and K Uptake by Rice
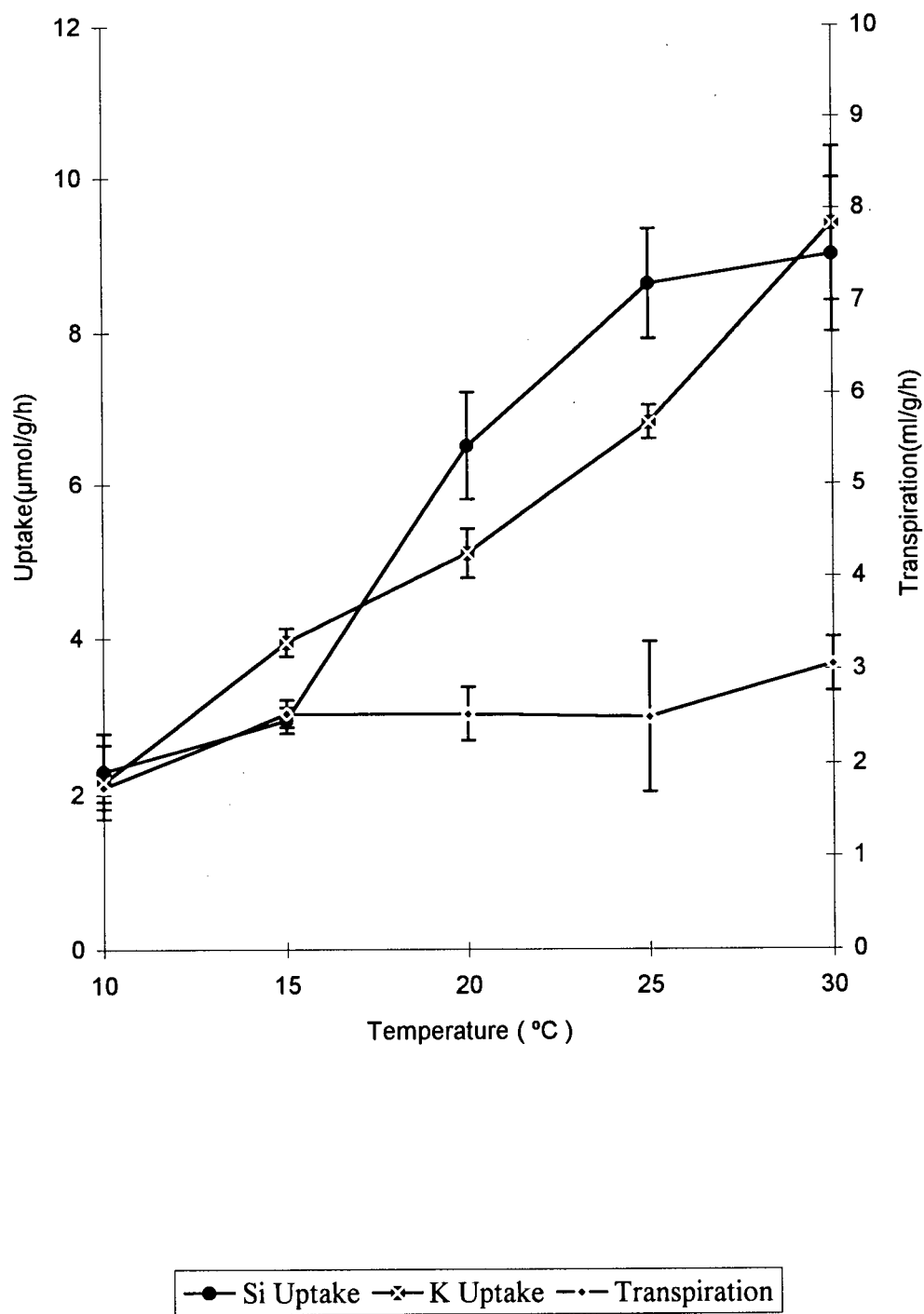
RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Temp. = 10, 15, 20, 25, 30 ° C

Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM

Samples every 30 min for 3 h.

Error bars represent 95% confidence limits.

Figure 7: Temperature Dependence of Silicon and Potassium Uptake by Rice

E. EFFECT OF ANOXIC CONDITIONS

Uptake of silicon from an anoxic solution was 66% less than uptake from an aerated solution (Figure 8). Similarly, potassium uptake was inhibited by 63% under anoxic conditions, which is in within the range of previously reported data (Thibaud et al., 1986; Brauer et al., 1987). Both Na^+ and K^+ uptake were inhibited by between 80 and 95% under anaerobiosis during the uptake period in barley (Brauer et al., 1987), as were rates of transport of these elements to the shoot. Transpiration, on the other hand, was not significantly affected by the absence of oxygen in the uptake medium. Although some metabolic activity continues in the absence of oxygen, ions that are actively accumulated by root cells require the products of aerobic respiration for transport, and therefore suffer depressed or completely inhibited rates of uptake under anaerobic conditions (Glass, 1988; Clarkson, 1986; Epstein, 1972). Phosphate uptake is inhibited by 50% under anaerobic conditions (Hopkins, 1956), while nitrate uptake is inhibited by 62% (Rao and Rains, 1976). The inhibition of silicon uptake under anaerobiosis further reinforces its dependence on energy from respiration. It is interesting, however to note that the response of silicon uptake to anoxia is less rapid than the response of potassium uptake to the same treatment (Figures 8a and 8b), while the magnitude of the response overall is similar. This perhaps implies that the dependence of silicon uptake on metabolic energy is less direct than that of potassium uptake.

<u>Figure 8: Silicon and Potassium Uptake by Rice Under</u>

<u>Control (Aerated) and Anoxic Conditions.</u>

Temp = 25°C, RH ~ 60%, LI ~ 500 μE.m^{-2}.s^{-1}

Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM

Samples every 30 min for 3 h

Error bars represent 95% confidence limits.

Percentages with each series represent the change in

transpiration or uptake upon the imposition of anoxic conditions.

The arrows accompanying those percentages represent the

direction of this change.

Figure 8: Silicon Uptake, Potassium Uptake, Transpiration, and Uptake /Transpiration Ratios in Rice Under Control (Aerated) and Anoxic Conditions

Figure 8a: Time Course of Silicon Uptake by Rice Under Control (Aerated) and Anoxic Conditions

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM

Samples every 30 min for 3 h

Error bars represent 95% confidence limits.

Figure 8a: Time Course of Silicon Uptake by Rice under Control (Aerated) and Anoxic Conditions

Figure 8b:Time Course of Potassium Uptake by Rice Under Control (Aerated) and Anoxic Conditions

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM

Samples every 30 min for 3 h
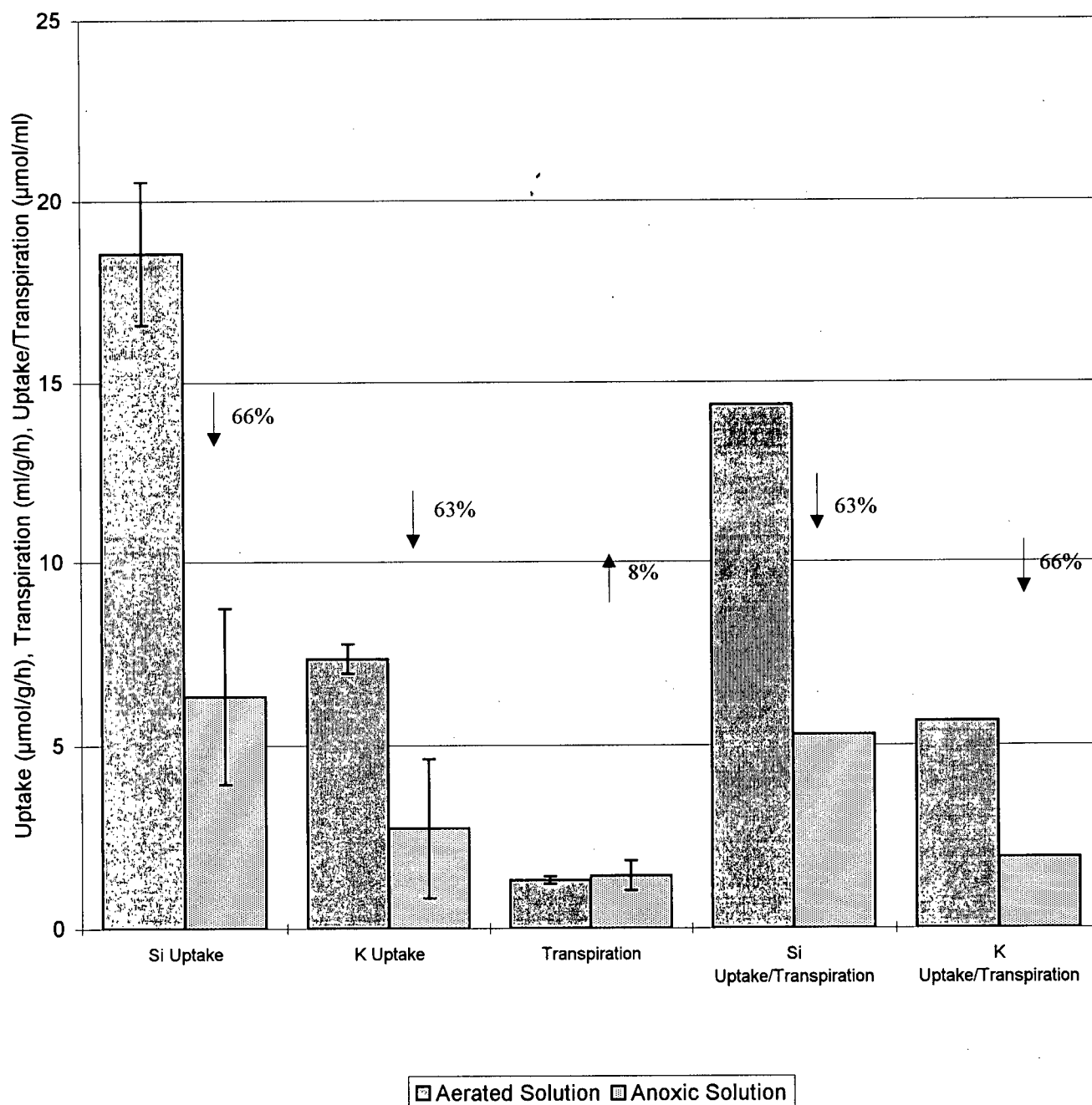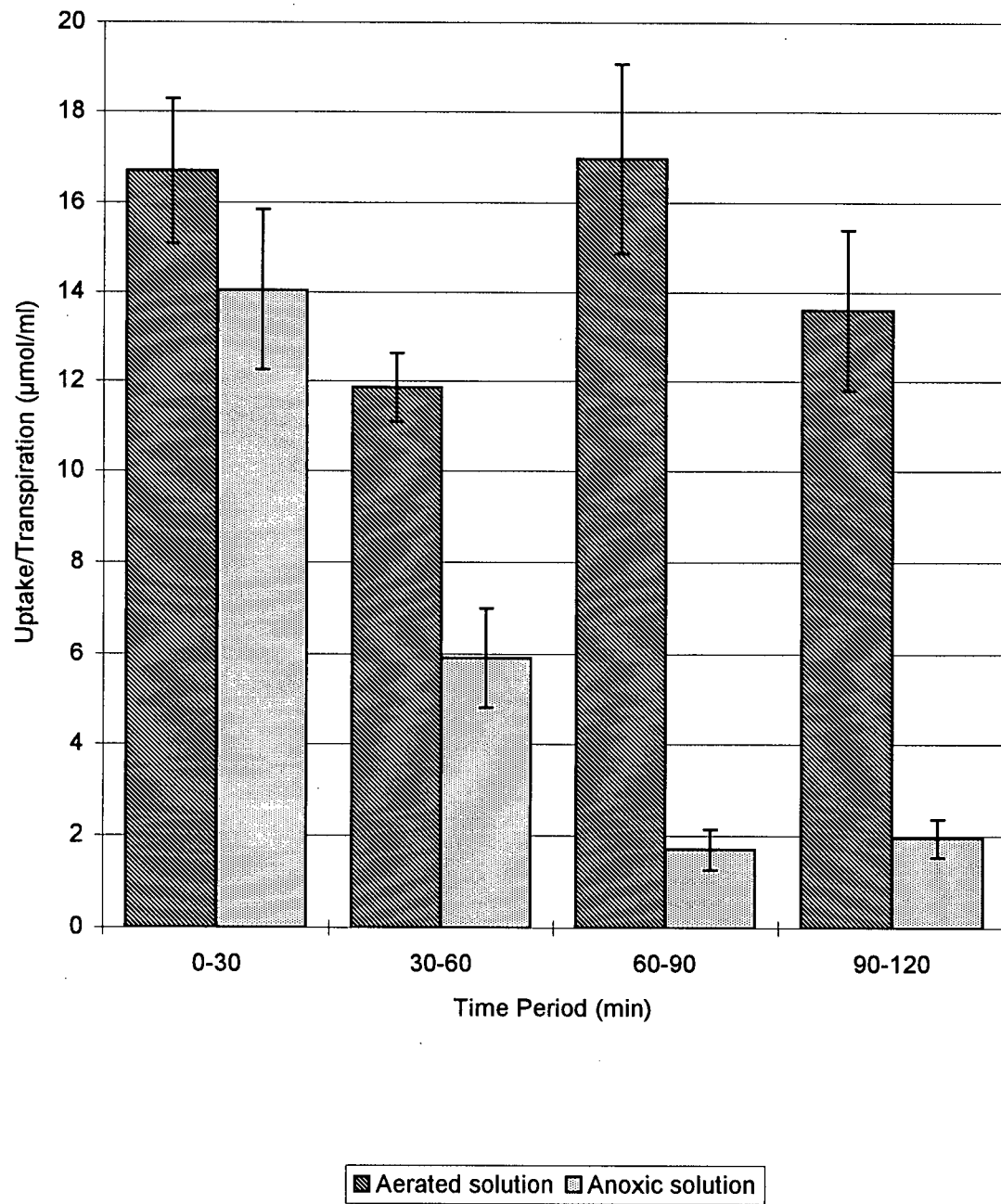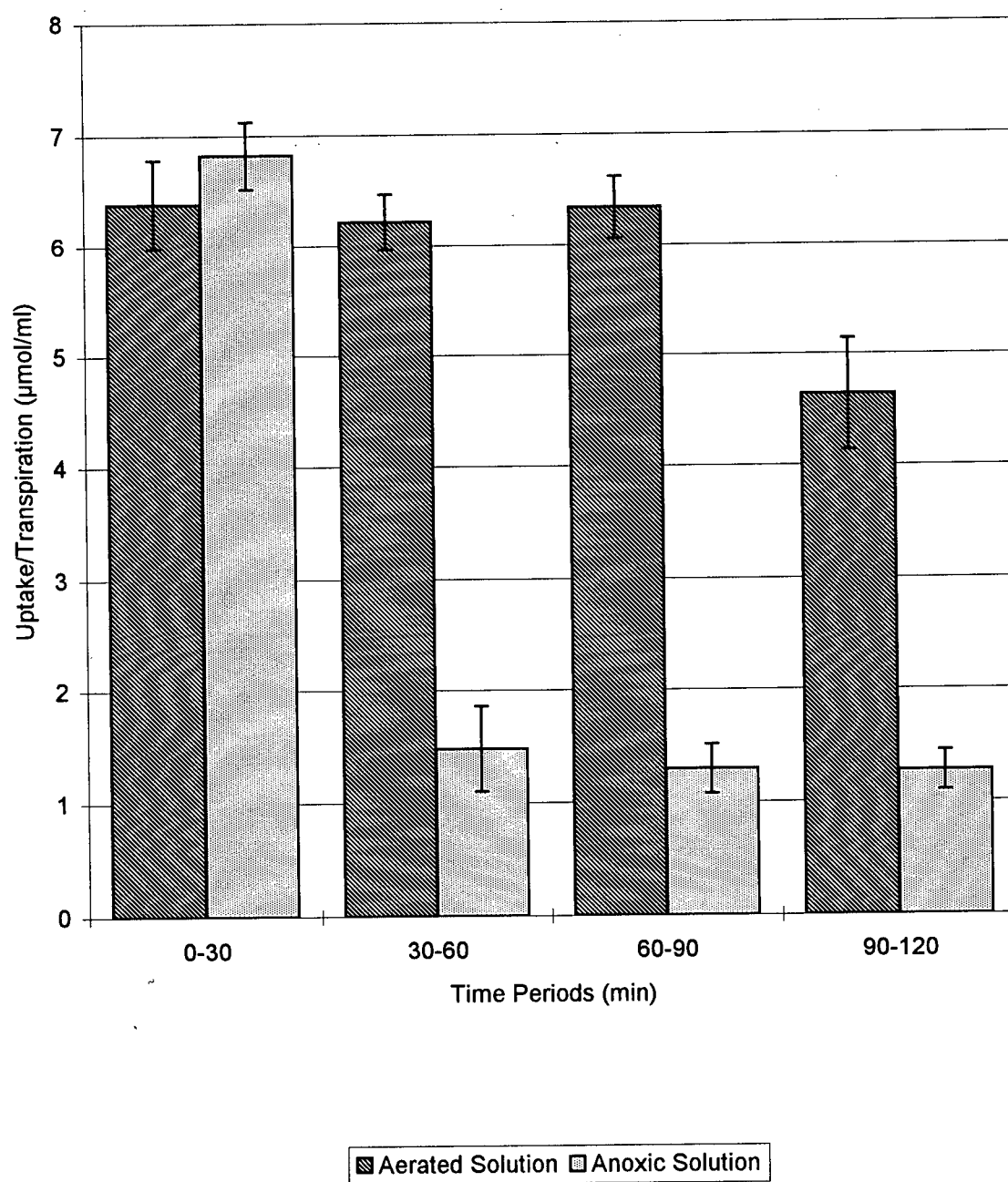
Error bars represent 95% confidence limits.

Figure 8b: Time Course of Potassium Uptake by Rice Under Control (Aerated) and Anoxic Conditions

F. EFFECT OF METABOLIC INHIBITORS

Treatment of the plant roots with KCN caused uptake of both potassium and silicon to decrease, yet potassium uptake was nearly 100% inhibited, while silicon uptake was only 80% inhibited (Figure 9), and the response of silicon uptake to exposure to KCN occurred more slowly than did the response of potassium uptake to the same treatment. Similar treatment with KCN was previously reported to decrease potassium uptake by 90%, and the decrease in K^+ influx was positively correlated with a decrease in cellular ATP levels, thereby establishing the dependence of K^+ influx on available ATP (Petraglia and Poole, 1980). The high affinity transport systems for both ammonium (Wang et al., 1993b) and nitrate (Glass et al., 1990a) have also been shown to be 75-87% inhibited by treatment with KCN. This would seem to imply that silicon uptake depends to a lesser degree on metabolic energy than does potassium uptake, yet this contradicts information described in the preceding sections, obtained at varying temperatures and under anaerobic conditions, which indicated a similar degree of metabolic dependence for both processes. It is possible that silicates, which are very prone to polymerization under various circumstances, were affected by the presence of cyanide and polymerized into a form that could not be absorbed by the plants yet could still be detected with the assay. In addition, the possibility exists that, like nitrate (Glass et al., 1990) and potassium (Petraglia and Poole, 1980), Si uptake is more sensitive to metabolic inhibitors at lower concentrations, and that at 0.1 mM Si, KCN inhibition of silicon uptake may be similar to that of potassium uptake at 0.1 mM K^+. However, it is also possible that silicon transport, though dependent on metabolic energy, does not directly depend on cellular ATP levels, but that a phosphorylated intermediate provides the energy for transport, as is the case for certain group translocation uptake mechanisms. Were a less direct dependence on metabolic energy the case for silicon transport, it would account for the slower, if not less pronounced, effect of metabolic inhibitors on uptake.

Figure 9: Time Course of the Effect of KCN on Si and K Uptake by Rice Plants

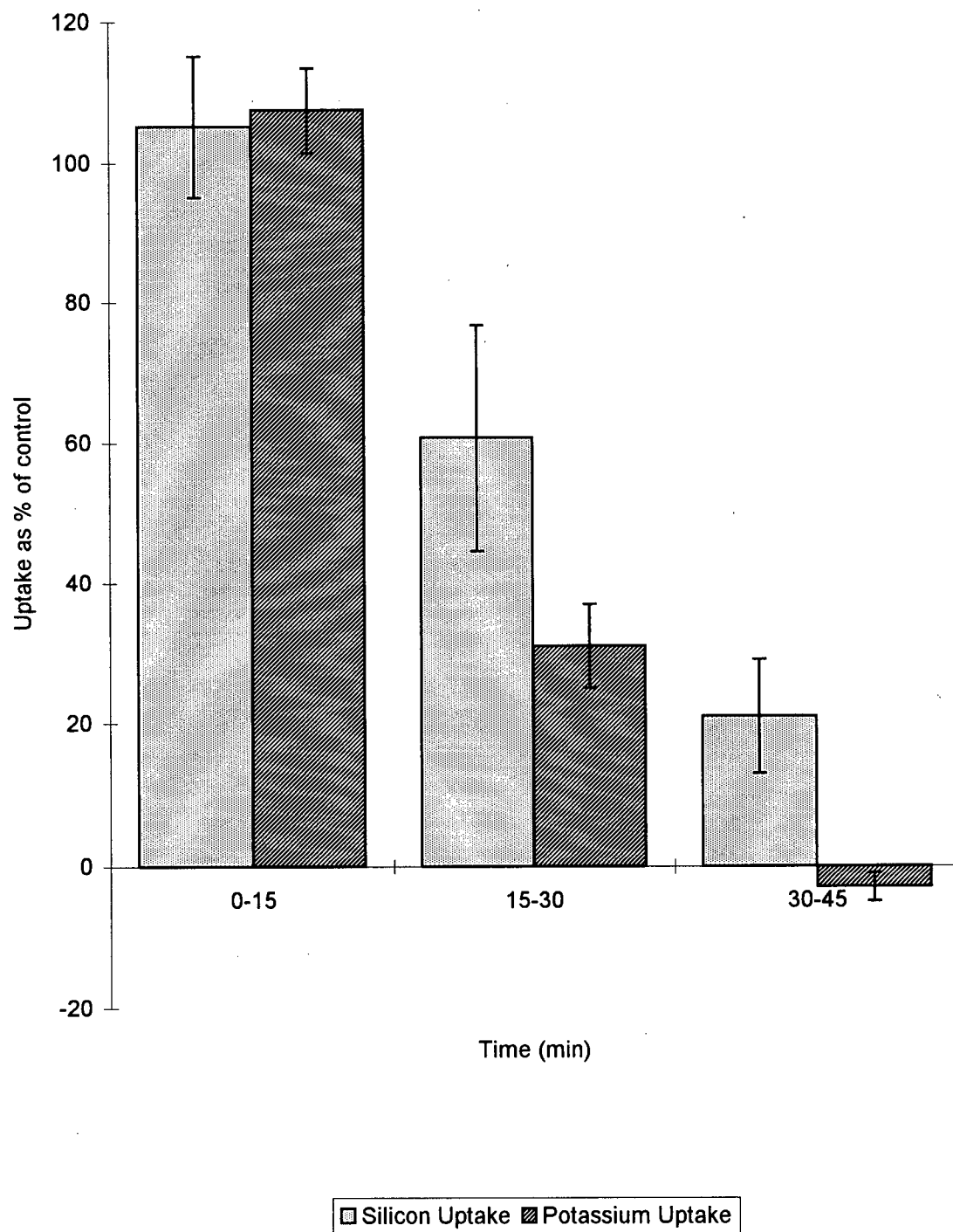Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0.5 mM, Initial [K] = 0.8 mM

[KCN] = 1 mM, [KCN]$_{control}$ = 0 mM

Samples every 15 min for 1 h.

Error bars represent 95% confidence limits.

Figure 9: Time Course of the Effect of KCN on Si and K Uptake by Rice Plants

G. EFFECT OF SILICON PRETREATMENT

Rice plants that were deprived of silicon during their growth period showed marked differences from those grown with 1 mM Si: their leaves were particularly soft and fragile, they showed a predisposition to wilting and their growth rate was visibly reduced, though this has yet to be experimentally demonstrated. The uptake of silicon by plants which had been deprived of silicon (-Si) was immediately enhanced compared to that of plants which had been grown with 1 mM Si (+Si) when they were reintroduced to a solution containing silicon. The enhanced rate of uptake in -Si plants was between 1.5 and 2.0 times higher than the rate of uptake in +Si (see Figure 10).

Fluxes of both potassium and ammonium have been shown to be similarly enhanced after periods of deprivation of these ions, though the extent of the increases were greater than was observed here for silicon. Glass (1976) established that a negative correlation exists between K^+ influx and root $[K^+]$. This is characteristic of a derepressible system whereby the internal concentration of the ion regulates its uptake by repression or derepression of a gene, by direct allosteric control of the transporter, or by a combination of the two. He postulated a system of allosteric control for K^+ influx, based on an analysis of the effect of root $[K^+]$ on K^+ influx rates and influx kinetics, without, however, discounting the possibility of gene repression having some role in long term regulation (Glass, 1976). The high affinity ammonium transport system of rice was also found to be regulated by negative feedback (Wang et al., 1993a); plants grown in low ammonium concentrations showed a higher V_{max} and lower K_m than plants grown in high ammonium when they were both subjected to the same conditions. The nitrate uptake system, on the other hand, exhibits a different pattern of regulation, in which plants grown in a nitrate-free medium exhibit initially low uptake rates which, after a lag of several hours, increase as exposure to nitrate lengthens. This is characteristic of an inducible system in which

exposure to the ion causes the induction of synthesis of the transporter (Glass, 1988; Glass and Siddiqi, 1994).

The silicon transport system in diatoms shows characteristics of a derepressible regulatory system similar to that observed for potassium in higher plants, as well as for silicon uptake by rice in this study. Si- limited cultures, showed immediately enhanced rates of uptake that then slowly declined after silicon was reintroduced into the culture medium (Harrison et al., 1989).

Whether or not a derepressible system is responsible for regulation of silicon uptake has yet to be determined, but the enhanced rates of uptake exhibited by -Si plants point to this as a possibility. Further study of the relationship between Si uptake and root [Si], as well as the kinetic parameters of plants grown at differing Si levels would be valuable in further elucidating this question.

<u>Figure 10: Effect of Si Pretreatment on Si Uptake by Rice</u>

<u>Plants</u>

Temp = 25°C, RH ~ 60%, LI ~ 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0.5, 1, 2 mM

Pretreatment [Si] = 0, 1 mM

Samples taken every 40 min for 4 h.

Error bars represent 95% confidence limits.

Figure 10: Effect of Si Pretreatment on Si Uptake by Rice Plants

Si Uptake (µmol/g/h)

External [Si] (mM)
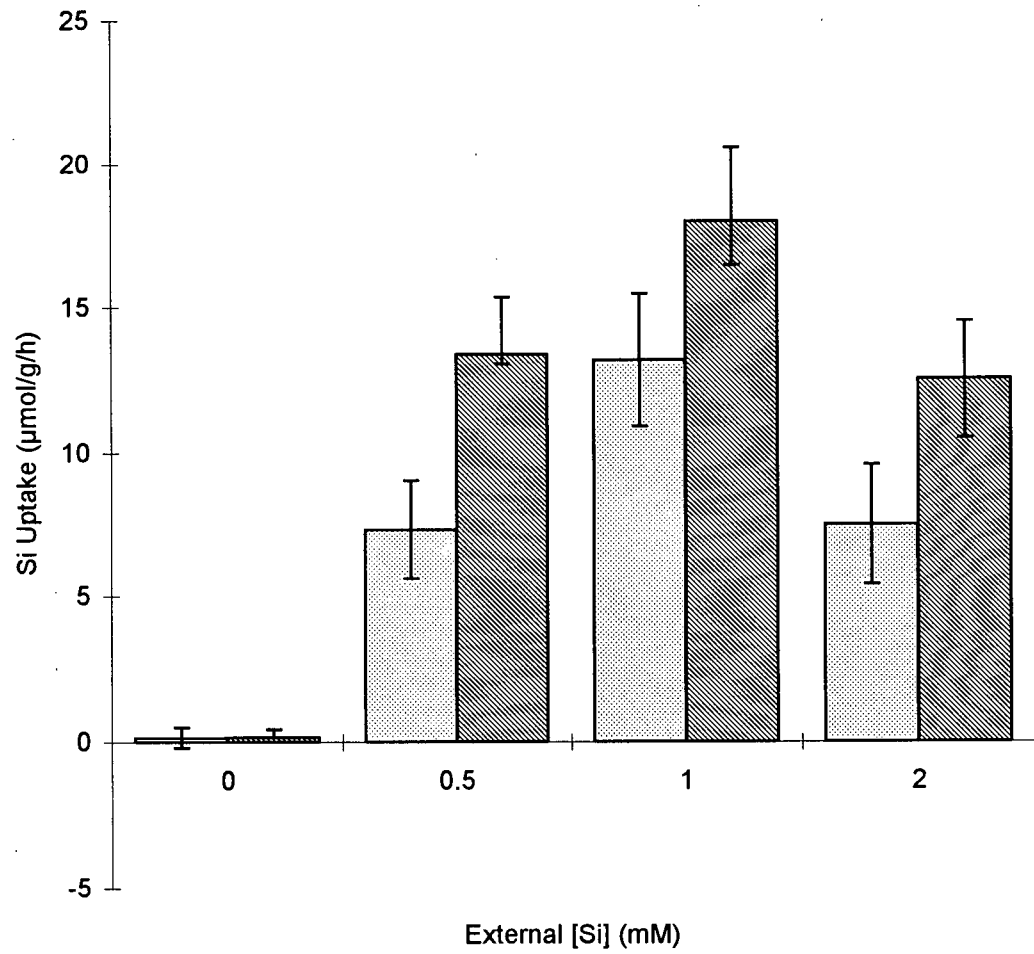
☐ Plants grown in 1 mM Si ▨ Plants grown in 0 mM Si

II. SILICON UPTAKE IN CUCUMBERS

A. TIME DEPENDENCE

Two experiments were conducted, which examined both time dependence and pretreatment (Figure 11). The plants fully depleted a 1 mM Si solution of Si after 32 to 48 hours. Initial uptake rates were between 5 and 9 μmol.g root^{-1}.h^{-1} and were relatively stable for the first four hours, after which they began to decrease. The average rates over the experimental period were 3.5 and 3.2, μmol.g root^{-1}.h^{-1} (Figure 11).

<u>Figure 11: Depletion of Si by Cucumber Plants as a Function of Time:</u>

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 1 mM

Samples taken every 4 h for 32 h

Figure 11: Uptake of Si by Cucumber Roots over Time
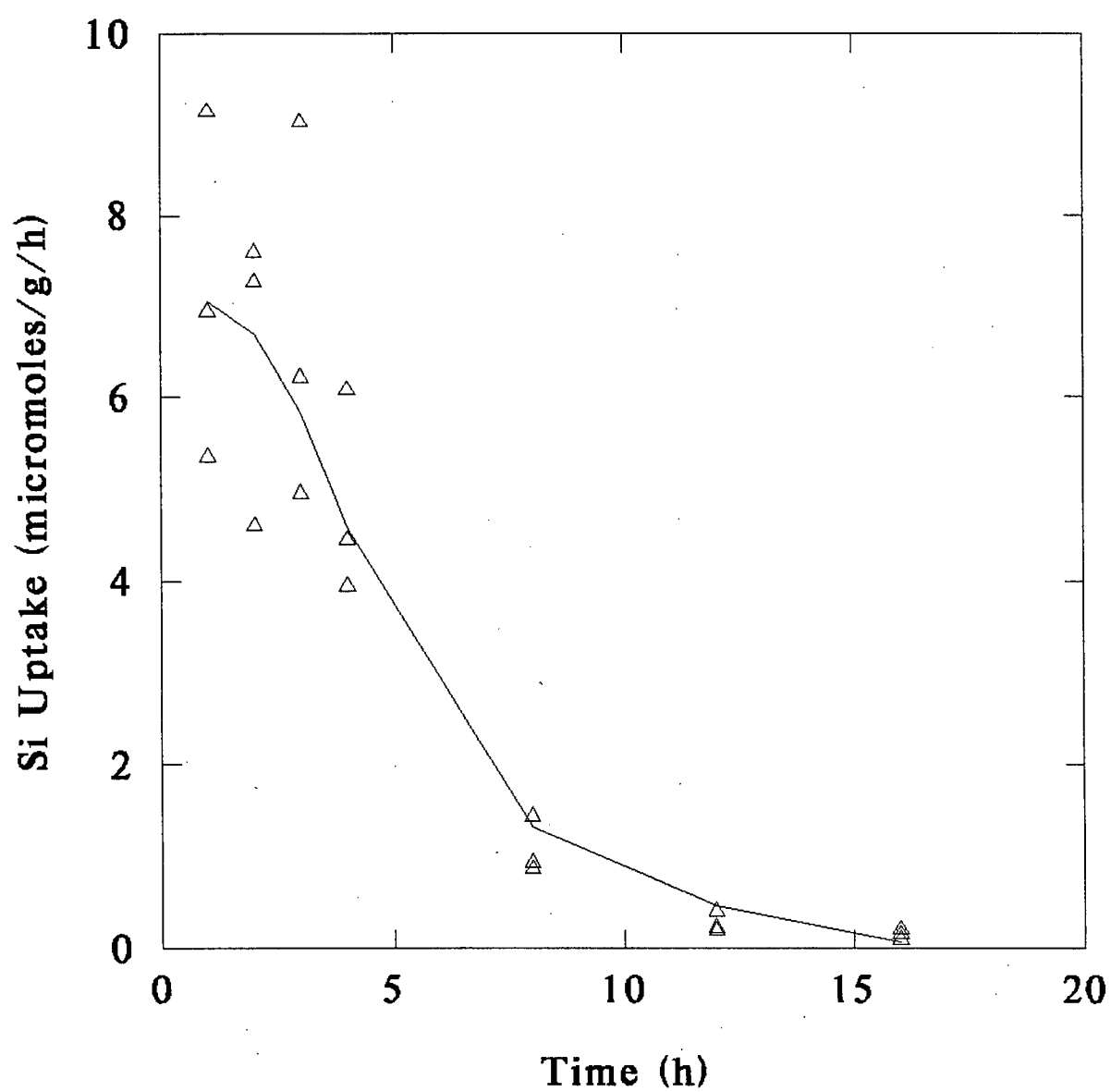
B. CONCENTRATION DEPENDENCE

The kinetic data for uptake of cucumbers can be interpreted in two ways. If the data are interpreted as representing the linear pattern of uptake characteristic of a passive flux (Figure 12), the r^2 value is 0.58 if the zero value is included, which makes the possibility of a purely linear pattern of uptake unlikely. However, if the zero value is not included, the r^2 is 0.965. It is possible, therefore, that uptake at higher concentrations is a passive process. If the data are interpreted as representing a saturating system (Figure 12a), which can be described by the Michaelis-Menten equation, a Hofstee plot of the data (Figure 12b) is linear with an r^2 of 0.829, suggesting a carrier-mediated system of uptake. This analysis points to a low affinity carrier-mediated system with a K_m of 0.835 mM and a V_{max} of 12.5 μmol/g/h. However, Figure 12a, appears to show a linear pattern of uptake above 2 mM external silicon. Perhaps a low affinity carrier-mediated system is responsible for silicon uptake within the range of concentrations that the plants are likely to encounter in soil, i.e. below 1.3 mM (Birchall, 1978), whereas at high concentrations silicon enters the plant passively.

Long term studies have shown some disagreement on the subject of passive or active silicon accumulation in cucumbers. Adatia and Besford (1986) found that cucumbers depleted silicon from culture solutions in which they were grown, and concluded that the plants were actively accumulating it. Takahashi et al. (1990), however, found that cucumbers caused no measurable change in the Si concentration of the culture solutions they were grown in, concluding that silicon uptake was occurring passively along with the transpiration stream. These workers recognize, however, that cucumber leaves can reach silicon levels as high as some graminaceous plants (Miyake and Takahashi, 1983b), and will suffer symptoms of silicon deficiency during the reproductive phase if they are grown without it (Miyake and Takahashi, 1983b). On the basis of the results obtained here, it is possible that a low affinity, saturable system is responsible for silicon

uptake. The low affinity of the system for it's substrate could explain why, under long term experimental conditions , a linear, bulk flow pattern of uptake was observed. It would be of interest to examine concentration dependence of silicon uptake in cucumbers in more detail at concentrations below 0.5 mM using a more sensitive method of Si detection such as a radioactive tracer, as well as to study the metabolic dependence of the system as was done here for rice.

Figure 12: Concentration Dependence of Si Uptake in Cucumbers

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 mM

Samples taken every 30 min for 4 h.

Error bars represent 95% confidence limits.

Line represents a linear fit: $r^2 = 0.58$ if zero value is included;

$r^2 = 0.965$ if zero value is excluded.

Figure 12: Concentration Dependence of Si Uptake in Cucumbers
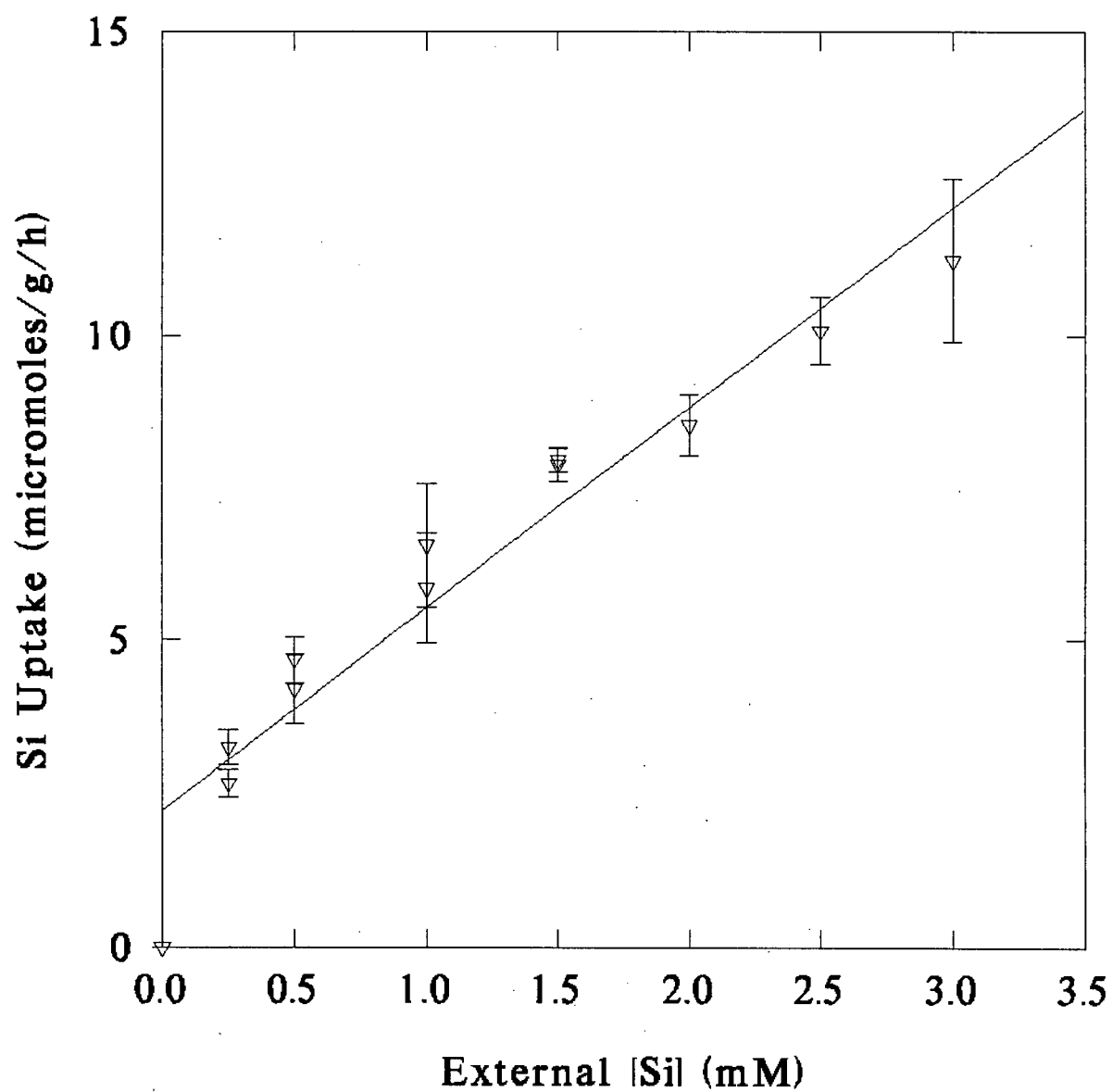
Figure 12a: Concentration Dependence of Si Uptake in Cucumbers

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 mM

Samples taken every 30 min for 4 h.

Error bars represent 95% confidence limits.

Line represents a Michaelis Menten fit as analyzed kinetically in figure 12b

Figure 12a: Concentration Dependence of Si Uptake in Cucumbers
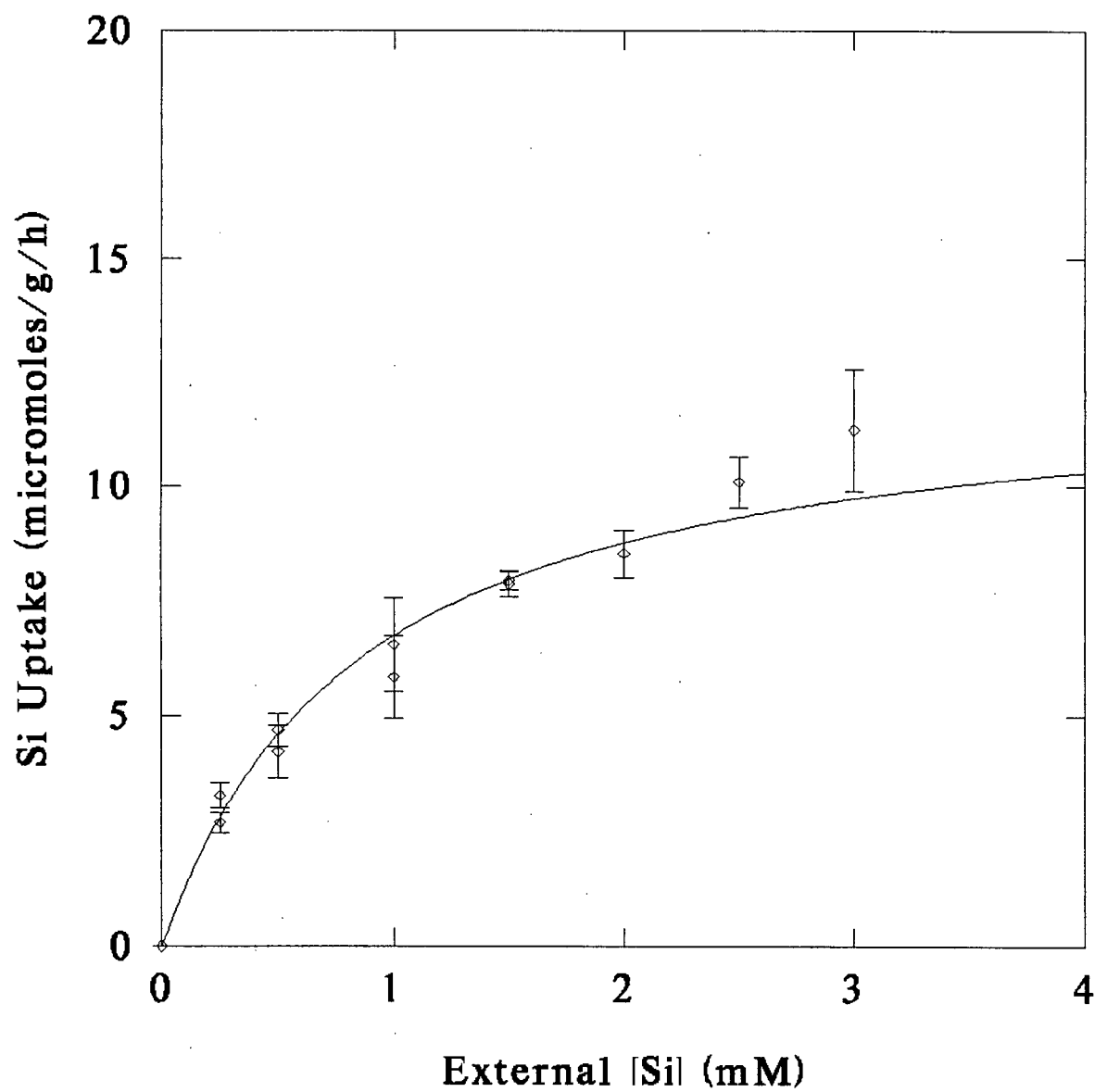
Figure 12b: Hofstee Plot of Si Uptake by Cucumbers

Data used are those represented in Figure 12a.

V represents uptake ($\mu mol.g^{-1}.h^{-1}$). [S] represents substrate concentration (mM).

$r^2 = 0.829$, $K_m = 0.835mM$, $V_{max} = 12.5$ $\mu mol.g^{-1}.h^{-1}$

Figure 12b: Hofstee Plot of Si Uptake in Cucumbers
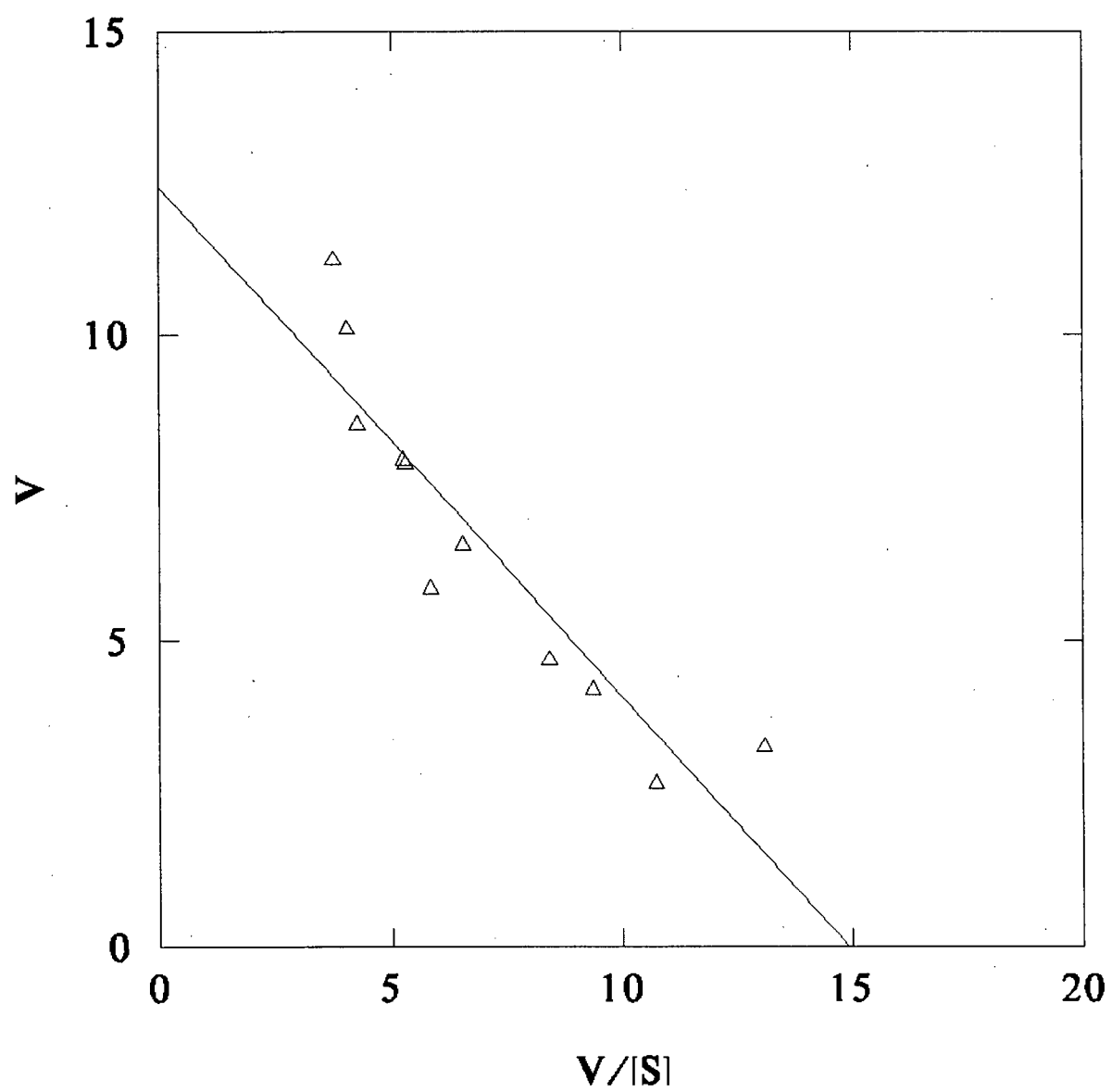
C. EFFECT OF PRETREATMENT

Two experiments were done, which looked at both time dependence and pretreatment. The rate of uptake and its pattern over time reflect no significant effect of Si pretreatment on uptake (Figure 13), though plants grown at 0 mM Si did show a slightly enhanced rate of uptake compared with plants grown at 1 mM Si. In addition, cucumber plants grown without silicon showed no discernible symptoms of silicon deficiency. It seems unlikely therefore that a derepressible system is responsible for regulation of silicon transport in this case. However, further study of the relationship between Si uptake and root [Si], as well as the kinetic parameters of plants grown at differing Si levels would be valuable in further elucidating this question.

<u>Figure 13: Effect of Si Pretreatment on Si Uptake by</u>

<u>Cucumbers</u>

Temp = 25°C, RH = 60%, LI = 500 $\mu E.m^{-2}.s^{-1}$

Initial [Si] = 1 mM, [Si]$_{control}$ = 0 mM

Pretreatment [Si] = 0, 0.5, 1 mM for a 2 week period.

Samples taken every 4 h for 32 h

Figure 13 : Effect of Si Pretreatment on Si Uptake by Cucumbers
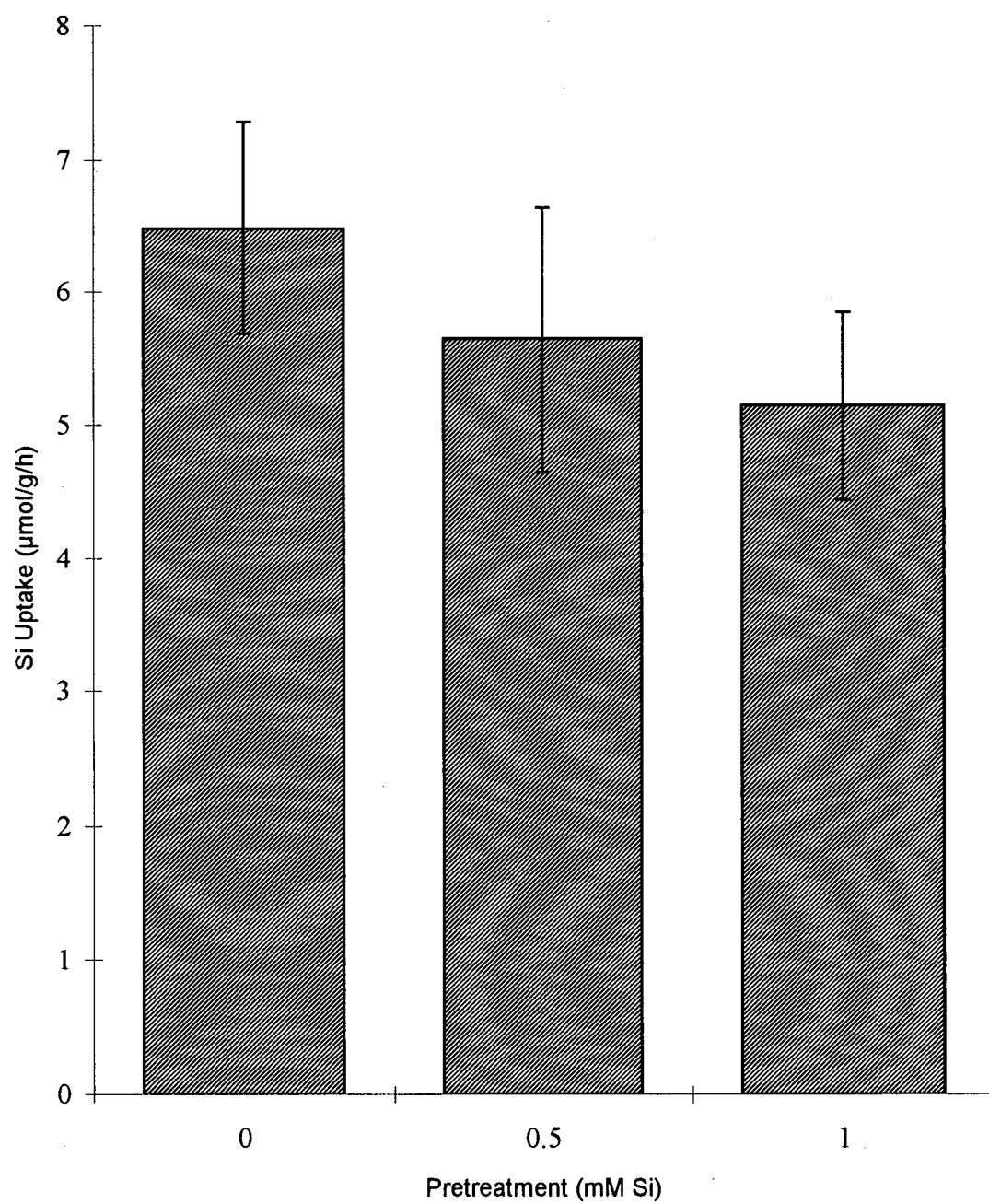
CONCLUSIONS AND PERSPECTIVES

The study of silicon uptake by rice indicates that an energy dependent, carrier-mediated system is responsible for transport, and that transport is independent of transpiration. The response of silicon uptake in rice to inhibitors of metabolism was, however, slower than the response of potassium uptake, though of similar magnitude over the experimental period. This raises the possibility that the reliance of silicon transport on energy from metabolism is less direct than it is for potassium transport, which depends directly on ATP supply and/or the proton motive force (Kochian and Lucas, 1988). Perhaps a previously phosphorylated intermediate, either membrane-bound or associated with a membrane-bound protein, provides energy for silicon transport. It is also possible that a method of transport similar to group translocation mechanisms in other organisms may be in operation, modifying the silicon as it is brought into the cell. The silicon transport system in cucumbers shows several characteristics that suggest it may also be a carrier-mediated system, though it is likely to be a very low affinity system.

The importance of silicon is already well established for proper growth and reproduction in many graminaceous species, and seems likely for many other species as well. In addition, the demands of hydroponically grown plants have highlighted the importance of silicon for all plant growth. Increased susceptibility of hydroponically grown crops to fungal infections has been remedied by adding silicon to the culture medium (Samuels et al., 1990; Takahashi et al., 1990). Epstein (1994) has suggested that Si be included in all solution cultures, as he considers that -Si plants represent experimental artifacts.He has further suggested that ample evidence now exists that silicon, when available to plants, "plays a large role in their growth, mineral nutrition, mechanical strength and resistance to fungal diseases, herbivory, and adverse chemical conditions of the medium". As the importance of silicon for plant growth becomes better established, it is important to understand more fully the physiology of silicon acquisition and utilization for the plant. This study has shown that the silicon transport system in rice, and perhaps that of cucumber as well, bears many resemblances to more thoroughly studied nutrient

transport systems, and could therefore be further examined using experimental methods employed to study these systems. The nature of the link between metabolism and silicon uptake could be further explored by assessing the effect of compounds that destroy the proton motive force or inhibit ATPases on silicon uptake. In addition, the use of microelectrodes to monitor any pH changes at the membrane surface associated with silicon uptake would provide useful information to elucidate the method of transport. Finally, by undertaking general kinetic and metabolic studies, such as those presented here, among a range of plant species it may be possible to elucidate a common mechanism of silicon uptake in all plants. The differing silicon contents and differing silicon requirements among species may result from and be reflected in the apparently widely different affinities for Si uptake in these species..

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