1994

Sand Accretion And Its Effects On The Distribution And Ecophysiology Of Dune Plants

John V. Perumal

SAND ACCRETION AND ITS EFFECTS ON THE DISTRIBUTION AND ECOPHYSIOLOGY OF DUNE PLANTS

by

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Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Ontario

June 1994

ISBN 0-315-93237-6

ABSTRACT

The effects of sand accretion on the lacustrine sand dune vegetation of Lakes Huron and Erie were studied. The tolerance and response to burial in sand of several dune species, namely, *Ammophila breviligulata*, *Andropogon scoparious*, *Agropyron psammophilum*, *Artemisia campestris*, *Calamovilfa longifolia*, *Cakile edentula*, *Cirsium pitcheri*, *Corispermum hyssopifolium*, *Elymus canadensis*, *Equisetum arvense*, *Euphorbia polygonifolia*, *Lithospermum caroliniense*, *Meliotus alba*, *Oenothera biennis*, *Panicum virgatum*, *Poa compressa*, *Strophostyles helvola*, *Tusilago farfara*, *Populus balsasmifera* and *Xanthium strumarium* were examined under controlled (greenhouse and growth chamber) and natural (field) conditions. This study clearly showed that the amount of sand deposition was variable in different microsites and depended on the amount of sand brought up by the waves, the wind velocity, the distance from the lake, type of vegetation, and the time of year. The distribution of plants in the dunes was correlated with the extent of sand accretion and tolerance limits of species. For example, *Ammophila breviligulata* was present in areas where there was more than 50 cm of sand movement (erosion and accretion) in two growing seasons. Simulated burial experiments in the field showed that although there were significant differences between species in their tolerance limits, all dune species exhibited stimulation of the net CO_2 uptake, leaf area, and biomass per plant at varying levels of burial in sand. The positive effects of burial were much more pronounced in the perennials, *Agropyron psammophilum* and *Panicum virgatum*, than in the annuals or biennials. Similar stimulation in growth following burial was also observed under controlled greenhouse and growth chamber conditions in all the species investigated. For example, in the greenhouse burial experiment *Elymus canadensis* plants buried to one third of their height had a CO_2 exchange rate of 28 μmol m^{-2} s^{-1} as compared to 18 μmol m^{-2} s^{-1} for control plants. There were marked differences between species in their response to the different depths of burial and the length of time after the burial treatment. Light intensities and temperature regimes also made significant differences in the carbon dioxide exchange rate under growth chamber

conditions Another well recognized aspect in plant communities is the occurrence of mulualistic association with soil fungi to form mycorrhizae. A field survey of the dune plant community along Lake Erie, revealed that a large majority of plant species were colonized by vesicuar-arbuscular mycorrhizal (VA-Mycorrhizae) fungi. With the exception of *Equisetum arvense* all 11 species had variable percentages of vesicular, arbuscular and hyphal colonization. Greenhouse experiments showed that although VA-Mycorrhizae enhanced the morphological and physiological responses in dune plants, There were not solely responsible for the enhanced growth exhibited by buried plants *Agropyron psammophilum* had a CER value of 15 μmol m^{-2} s^{-1} for unburied plants containing VA-Mycorrhizae and 18 μmol m^{-2} s^{-1} for VA-Mycorrhizae-free plants that were buried and 25 μmol m^{-2} s^{-1} for buried VA-Mycorrhizae containing plants.

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my sincere gratitude to a number of individuals who have helped me with my research project and the writing of this thesis.

First, I am especially grateful to my supervisor, Dr. M. A. Maun, for his instructions, encouragement and financial support throughout this project. I appreciate the willingness on his part to assist with the many steps that led to the completion of this thesis. I want to thank him for his patience with me and his untiring drive through the various stages of writing this thesis.

I would like to thank my advisors Drs. N. P. Huner and J. A Traquair for their valuable advice and the suggestions given to improve my research project.

I especially would like to thank my family for their support through the 3 years of my study. My wife, Celine, for the love and help she gave me throughout the endeavour. My daughter, Veliska and my son, Viren for putting up with me during the course of my study. I am extremely indebted to them for their patience and understanding while I spent my evenings and many Sundays in the lab. I also want to thank the following individuals: Michael Mendis, for the many hours he spent helping me with the production of this thesis; Dr. R. C. Jancey and Madhur Anand, for their assistance with the statistical analysis of my data; Dr. J. Zhang, for his review and comments on certain parts of this thesis; Marisa Martinez, Richard Little, Andraya Gillen and Andrew Walde, for field assistance; Theresa Donworth and Daphne Boyce, for providing assistance in the greenhouse; Dr. F. Marks, of Agriculture Canada, for allowing me the use of the walk-in growth chamber; Drs L. Peterson and T. McGonicle, of the University of Guelph, for their assistance with the mycorrhizal work; Ron Smith, for help with microscopy in the mycorrhizal work; Donna Cheshuk, Stefani Tichbourne and Tracey Toso, for putting up with me during the three years when I needed help at the department office; Alan Noon and Ian Craig, for their assistance with photography; Don Yakobchuk, for being so helpful with providing the

supplies needed for the research; Erika Mueller, Monica von Dehn, Ming Zhan, Jackie Chernys, Steve Wilhelm, Angelo D'Ulisse, Tracy Phillips, and Mariana Król, for their help and friendship.

I would like tvio thank the Ministry of Natural resources for allowing me free access to the study sites at Port Burwell and Pinery Provincial Parks.

I also thank the Biology Department of Southern College in Collegedale, Tennessee, for giving me the opportunity of working for them upon completion of my studies.

At this point a few individuals not directly connected with my studies must be mentioned. Mrs. Edith Willis was the inspiration behind my undertaking such a daunting task. Greg and Rita Mogenson and Ruth Mogenson provided moral support and encouragement. Pastor Amorin and the members of the North London Seventh-day Adventist Church gave me their warmest support and fellowship through this phase of my life.

DEDICATION

This Ph.D. thesis is dedicated to Pastor Richard C and Mrs. Jean M. Hall for their dedication and service to my place of birth, Sarawak, in the island of Borneo The twelve years they spent there were years that made concrete impressions in the lives of many they came into contact with They have given me the one thing that has meant the most in my life--a friend in Christ Jesus. Their selfless and untiring lives have always been an inspiration in my life and I trust that the Lord will continue to give them the strength to do what they have always done for Him. I am grateful to the Lord for placing them in my path

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respiratory losses, is determined by irradiance, CO_2 and O_2 concentrations, and temperature, and is species dependent. Net photosynthesis (Pn) is the difference between Pg and the rate of CO_2 loss by photorespiration (Rl), and dark respiration (Rd) Pn = Pg - (Rl + Rd) The ratio of Pg to (Rl + Rd) is not constant but is species and environment dependent Dark respiration is the only type of respiration in the dark and it dominates at low irradiance but Rl is greater than Rd during assimilation with high O_2/CO_2 ratios (Lawlor, 1987)

There are a number of characteristics that distinguish C_3 from C_4 plants. C_3 plants are generally temperate species, are moderately productive and have leaves which do not show Kranz-type anatomy They generally lack peripheral reticulum In C_3 plants there is only one type of CO_2 fixation pathway, and the initial CO_2 acceptor is ribulose bisphosphate (RuBP), a 5-carbon sugar The initial CO_2 fixation product is the 3-carbon acid phosphoglycerate. C_3 plants are also known to have a low water use efficiency and salinity tolerance Photosynthesis saturates in most C_3 plants at 1/5 full sunlight and their stomata are open at night

C_4 plants on the other hand are typically tropical species, and the plants are highly productive The plants are adapted to high light intensities, high temperatures and semi-arid environments C_4 plants have a Kranz-Type anatomy and possess peripheral reticulum, both of which are important features. The initial CO_2 acceptor is phosphoenol pyruvate (PEP), a 3-carbon acid and the initial CO_2 fixation product is a 4-carbon acid oxalo-acetate, thus referred to as C_4 plants.

Photosynthetic capacity is the light saturated rate of CO_2 uptake, while F_v/F_m is the photochemical efficiency of the photosystem II

1.2 Test Species

A brief description of the common dune plant species used in this research project is given below

(1) *Agropyron psammophilum* Gillett & Senn (Great Lakes Wheat grass)

This plant is limited to the shores of Lakes Michigan and Huron. It is the characteristic grass of the dunes at Clark Point on the southern boundary of Bruce

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Literature Review

1.1.1 Dune Substrate Characteristics

The beaches of the Great Lakes have variable sand texture depending on beach exposure and sediment source (Maun and Baye, 1989) On a beach along Lake Huron, the freshly deposited material on the mid-beach ranged from 0.05 to >2.00 mm in diameter (Table 1 1) Wind sorts sand particles and causes differential eolian transport of grains of different sizes Pebbles and coarse gravel do not move inland and may act as a covering to prevent further deflation (Bagnold, 1941). The proportion of coarse particles is higher on the upper beach (Cowles, 1899). In contrast, the texture of sand on dune ridges is typically finer than the parent beach sand (Komar, 1976). Sands of coastal and lacustrine dunes are often deficient in nutrients Nitrogen, phosphorus, and potassium are the three macro-nutrients that are usually in short supply compared to the biological demand (van der Valk, 1974; Willis and Yemm, 1961)

Coastal dune soils for example, contain nitrogen concentrations of only 0 006-0.02% by weight in Britain (Willis *et al.*, 1959a,b) and 0.003-0.001% along the Pacific Coast (Holton, 1980). Phosphorus and potassium levels in dune soils may also be as low as 0.11 (Willis *et al.*, 1959a,b) and 0.008% (Maun, 1985), respectively. However, the prevalent nutrient status may not strictly represent their availability to plants The high permeability and low cation exchange capacity of sand favors rapid flow of nutrients following rainfall and decomposition of litter (Ralph, 1978) The primary source of nutrients for plants on the beaches along the Great Lake shores is wave deposited debris such as the macroalgae *Fucus vesiculosus* L., *Ascophyllum nodosum* L., *Alaria* and *Laminaria* spp. and other organic debris (Chapman, 1976). These macroalgae have a low carbon-to-nitrogen ratio of about 15:1 compared with over 300:1 for *Ammophila* spp leaf litter and 85:1 for *Spartina* spp (Maun and Baye, 1989) The deposition of debris is not uniform and the beach habitat may vary from

Table 1.1: Mechanical Composition (%) of Sand Samples Collected from the Midbeach, Highbeach, First Dune Ridge, and Slack of Lake Huron Sand Dunes*

Particle diameter (mm)	Percentage by weight			
	Mid-beach	Upper beach	First dune ridge	Slack
>2	6.9	0	0.02	21.5
0.5—2	24.0	3.4	0.2	28.9
0.25—0.5	35.0	63.6	6.9	44.2
0.21—0.25	20.0	22.2	17.3	3.4
0.14—0.21	14.0	10.2	74.6	1.6
0.05—0.14	0.1	0.6	0.9	0.4
<0.05	0	0	0.1	0

* Particle size classification: gravel = 1–2 mm; coarse sand = 0.6–1.1 mm; medium sand = 0.2–0.6 mm; and fine sand = <0.2 mm.

Adapted from Maun and Baye, 1989.

extremely deficient to highly enriched in nutrients over short distances (Maun and Baye, 1989) Nutrient imput by animals is poorly studied in sand dunes Ranwell (1972) reported that in areas where gull colonies gather and nest, guano rich in phosphorus and nitrogen may be deposited in substantial quantities Ants and other insects and large mammals may also play an important role in localized habitats (Ralph, 1978).

Nutrients released from turtle eggs have been shown to be used by roots of *Ammophila breviligulata* on a highly localized scale (Lazell and Auger, 1981). Since the sloping foredunes restrict the deposition of debris, the foredunes are extremely low in nutrients (Hawke, 1987; Ralph, 1978; and Willis, 1965) Here, the atmospheric inputs through bulk precipitation, fog, and oceanic spray may supply substantial quantities of certain nutrients (Maun and Baye, 1989) Estimates of annual nitrogen input through rain were 6.4 kg per hectare in the northeastern United States (Pearson and Fisher, 1971 as cited in Maun and Baye, 1989). Moisture availability is also quite variable on the beach and foredunes depending on the climate, soil organic matter, and beach geomorphology (Maun and Baye, 1989). Annual precipitation in the Great Lakes region ranges between 90 and 120 cm per year. The sandy substrate of the first dune ridge along Lake Huron had a mean field capacity of 10.65% but the moisture retention capacity was rather low (Baldwin and Maun, 1983).

1.1.2 Sand Dune Vegetation

The development of the Great Lakes sand dune systems and their vegetation has been described by several authors (Cowles, 1899, Olson, 1958a,b; Baldwin and Maun, 1983). The beach is referred to as the part of land that extends from the water's edge to the base of the first dune ridge (20-30 m) Therefore, it is subjected to frequent erosion and deposition of the substratum by wind and wave action (Cowles, 1899) The region closest to the water (low beach) is washed by waves continually and is without any vegetation The mid-beach, the region between the low beach and driftline, is highly influenced by the lake level and occurrence of winter storms and varies from year to year. During the summer months, the high beach (portion of beach beyond the action of normal waves), driftline, and mid-beach are usually colonized by

a sparse cover of annual and biennial plants (Maun, .993).

1.1.3 The Fauna of Sand Dunes

The many types of habitats within the sand dune system provide various suitable niches for a variety of invertebrates, birds, reptiles, and mammals. Ecological analysis has been done on only a few of the many invertebrate fauna in the dune complex (Willis, 1989). Invertebrates characteristic of this region are beetles, millipedes, centipedes, woodroaches, and many species of wood snails (Maun, 1993). A total of 261 species of birds has been recorded, of which 67 species are known to breed within the sand dune system (Maun, 1993) Bird species found on the beach and open dunes are gulls, eastern kingbird, swallows, brown thrasher, and morning warbler (Shelford, 1977) There has been a significant decrease in certain bird populations primarily due to deer browsing of shrubs and lower branches of young trees (A. Maun, pers. comm.) Two species of snakes occur in the open areas of the Great Lakes dunes. The common mammals found in the dune systems are chipmunks, mice, voles, and raccoons. The white tailed deer is found in all the habitats, while the beaver is found commonly along the rivers.

1.1.4 Mycorrhizal fungi in the Sand Dunes

Mycorrhizal fungi may facilitate absorption of nutrients, especially phosphorus, in dune systems. The extent to which nutrient uptake in dune plants is mediated by vesicular-arbuscular (VA) mycorrhizae is not yet known, but is presumably significant for phosphorus nutrition of established populations. Several common dune species are known to be colonized by VA mycorrhizae. The common VAM species are *Glomus* and *Gigaspora* spp (Koske, 1984). Diazotrophic bacteria (Azotobacter) associated with the roots of some dune plants fix substantial amounts of nitrogen. Estimates revealed that about 76% of the nitrogen content utilized by the plant could be accounted for by direct rhizosphere nitrogen-fixation of *Ammophila breviligulata*, during the current season (Maun and Baye, 1989) These organisms are an important part of the sand dune systems Mycorrhizae potentially affect all aspects of functioning of terrestrial

biomes from carbon allocation to nutrient mobilization Mosse (1975) has suggested that mycorrhizae not be considered as a plant-fungus interaction but as a plant-fungus-soil partnership. Soils, mycorrhizal fungal types, and host plants change depending on their physical environment Read (1983) has constructed a model of relationships among biomes, limiting responses and mycorrhizal associations In this model the mycorrhizal groups are fitted to physiology and structure, which regulate their ability to obtain resources and regulate production both for improved plant growth and carbon for the fungus's own requirements. Read (1993) suggested that VA mycorrhizae were an important driving force in plant community organization

1.1.5 Physiology of Dune Plants

Carbon dioxide, the major substrate for photosynthesis, is supplied by the environment in which an organism lives It is obtained from water by aquatic organisms and from the atmosphere by terrestrial plants Carbon dioxide concentration in the earth's atmosphere changes from year to year and with season, whereas oxygen concentration varies little. The world's atmosphere contains 7×10^{14} kg of carbon as CO_2 and there is 1×10^{15} kg of carbon in organic materials both living and dead in the biosphere (Lawlor, 1987).

The general net equation for photosynthesis can be written as follows

$$nCO_2 + nH_2O \text{ ·····light-----→ } n(CH_2O) + nO_2$$

This equation demonstrates the central role of light-dependent gas exchange, the assimilation of CO_2 and the evolution of O_2 during photosynthesis. The rate of photosynthesis by the whole leaf and its response to environmental conditions differs greatly between species of plants and is correlated with the habitat.

Photosynthesis in plants depends on (i) the rate of NADPH and ATP production; (ii) rate of synthesis of RuBP which is controlled by the PCR cycle, (iii) rate of carboxylation of RuBP which is a function of the activity of the RuBP carboxylase enzyme and of the ratio of RuBP oxygenase to carboxylase activity (i e of photorespiration to gross photosynthesis); and (iv) rate of supply of CO_2 to the enzyme activity sites (Lawlor, 1987)

Rate of gross photosynthesis (Pg), which is the rate of CO_2 assimilation before

respiratory losses, is determined by irradiance, CO_2 and O_2 concentrations, and temperature, and is species dependent. Net photosynthesis (Pn) is the difference between Pg and the rate of CO_2 loss by photorespiration (Rl), and dark respiration (Rd) Pn = Pg - (Rl + Rd) The ratio of Pg to (Rl + Rd) is not constant but is species and environment dependent Dark respiration is the only type of respiration in the dark and it dominates at low irradiance but Rl is greater than Rd during assimilation with high O_2/CO_2 ratios (Lawlor, 1987)

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(i) *Agropyron psammophilum* Gillett & Senn (Great Lakes Wheat grass)

This plant is limited to the shores of Lakes Michigan and Huron. It is the characteristic grass of the dunes at Clark Point on the southern boundary of Bruce

County and of the dry shore sands as far north as Dorcas Bay at the tip of the Bruce Peninsula Its distribution continues across Lake Huron to Manitoulin Island and Great Duck Island It is a C_3 plant

(ii) *Ammophila breviligulata* Fernald (American beachgrass)

A characteristic sand binder on beach dunes It occurs along the Atlantic Coast as far north as Newfoundland and along the shores of the Great Lakes In Ontario A breviligulata are somewhat localized and do not occur on all sand dunes along the Great Lakes It is a C_3 species

(iii) *Andropogon scoparius* Michx (Little bluestem, Prairie beard grass)

A characteristic prairie grass, largely restricted in distribution in Ontario to the sand dunes and dry rocky shores of Lake Huron, Lake Erie, and the Ottawa River It is a C_4 tufted perennial with solid, hard, and brittle culms

(iv) *Arctostaphalos uva-ursi* L (Spreng (Bearberry)

A prostrate shrub forming mats up to a meter wide Leaves are coriaceous, evergreen, lanceolate to oblong-obvate, 1-3cm long, obtuse or rounded, entire, tapering to the base It is a C_3 perennial which is commonly found in sandy and rocky soils.

(v) *Artemisia campestris* L (Wormwood)

A biennial or monocarpic perennial with a taproot and generally several glabrous to vilous stems from a branching caudex, commonly 10-30 cm tall. Basal leaves crowded, about 2-10 cm long including the petiole, 0 7-4 cm wide, twice or three times pinnatifid or ternate, with mostly linear or linear-filiform divisions It is found in open areas, especially in sandy soil. It is a C_3 plant

(vi) *Cakile edentula* (Bigel) Hook var *lacustris* Fernald (Sea rocket)

An annual plant of the family Brassicaceae It is a low-growing herbaceous plant of beaches, usually growing 15 to 30 cm tall. Generally bushy and much branched, the plants have fleshy leaves and produce small, pale, pink/purple flowers (Hawke and Maun, 1988) The plant is generally found on sandy or gravel beaches and on the sea coast from Labrador to South Carolina in North America (Fernald, 1950) In Ontario this C_3 plant is well distributed on the beaches and shorelines of all the Great Lakes It is a C_3 species

(vii) *Calamovilfa longifolia* (Hook.) Scribn. (Prairie sand reed grass)

A characteristic perennial grass of the drier prairies Coarse, grass with hard scaly rhizomes Leaf blades glabrous, coarse, broad at base but tapering into a long thin tip. It is an important sand binding C_4 plant along the Great Lakes.

(viii) *Cirsium pitcheri* Torr T. & G. (Pitcher's thistle)

This plant is endemic to the western Great Lakes region where it is restricted to the sand dunes along the shoreline (Loveless, 1984). The plant is found in both open and more stabilized dune areas, and in both habitat types they establish in very open, sandy soil The plant is considered to be threatened in most of its range due to increased recreational activities (Keddy, 1987). It is a C_3 species

(ix) *Corispermum hyssopifolium* L. (Bugseed)

An annual C_4 plant of the family Chenopodiaceae. The plant is highly branched and fleshy, and grows up to a height of 60 cm. It is distributed in North America from Quebec in the East, to Washington in the West, to Indiana in the South on sandy beaches, dunes, and openings (Fernald, 1950).

(x) *Elymus canadensis* L. (Canada Wild-rye)

It is a tall, coarse, native grass common along the dry sandy shores of the Great Lakes from sand banks in Prince Edward County to Thunder Bay. This grass is a biennial and does not spread by rhizomes. Considerable variation exists in its shape and habit. It belongs to the family Gramineae. It is a C_3 species.

(xi) *Equisetum arvense* L. (Field horsetail)

It is a perennial which has no flowers or seeds but reproduces by spores and underground rhizomes It occurs in all parts of Ontario in low-lying areas with poorly-drained soils as well as in sandy or gravelly soils with good drainage. It is a C_3 species.

(xii) *Lithospermum carolinense* (Walt) MacMill. (Gromwell)

A perennial herb, native to North America and is usually found in sandy soils. The species is characterized by erect stems arising from a woody taproot containing a purple to red staining dye The alternate, linear to lanceolate leaves are covered with stiff, white hairs It has bright orange-yellow, funnelform flowers.

(xiii) *Melilotus alba* Desr. (White sweet-clover)

An annual or a biennial plant. Its taproot penetrates deep into the soil, and lateral roots are well developed with a large number of nodules It grows in a variety of communities ranging from agricultural land, roadsides, quarries, railway embankments, riversides, wastelands, and sand dunes The plant thrives in open areas and does not persist in shady areas It is a C_3 species

(xiv) *Oenothera biennis* L. (Evening primrose)

A biennial which is 1-2 m tall Flowers several to many in a terminal raceme, the bracts are resembling the leaves but much smaller It is found in fields, roadsides, and waste places throughout North America It is a C_3 species

(xv) *Panicum virgatum* L. (Switch grass)

A rather coarse perennial, usually without hairs, and it spreads in large clumps by short, hard, sharp pointed rhizomes It is distributed on the beaches of Bruce County and around Grand Bend on Lake Huron and at Point Pelee, Rondeau Park, Long Point, and Port Burwell on Lake Erie. It is a C_4 species

(xvi) *Poa compressa* L. (Canada blue grass, wire grass)

A very common and widespread perennial grass in all of southern Ontario It is readily recognized by its clean rhizomes, flattened stems with conspicuous nodes, and short, glaucous, or purple-tinged leaves.

(xvii) *Populus balsamifera* L. (Balsam poplar)

It is a tree, with long, straight, cylindrical trunk with a narrow open crown of a few stout, ascending branches, and a shallow root system. It grows across Canada throughout the Boreal, Great Lakes-St. Lawrence, and Acadian Forest regions

(xviii) *Strophostyles helvola* (L.) Ell. (Beach bean)

It is an indeterminate, herbaceous, warm-season annual of the family Fabaceae The plant has pinnately 3-foliate leaves with petioles. In North America the species is distributed throughout the eastern and central United States, westward to northern Mexico, and in humid areas of the northwestern United States and California In Ontario it is distributed mainly along Lake Erie. It is a C_3 species.

(xix) *Tussilago farfara* L. (Coltsfoot)

A perennial growing from a creeping rhizome Stems are about 5 cm tall, thinly tomentose, the bracts distant or crowded, about 1cm long Leaves long, petioled, cordate to suborbicular with deep narrow sinus, callous-denticulate and shallowly lobed, usually 5-20 cm long and wide, glabrous above, persistently white tomentose below. Native of the Old World, naturalized in disturbed areas, waste places, and sand dune beaches. It is a C_3 species.

(xx) *Xanthium strumarium* L (Cocklebur)

An annual weed which ranges from 2-20 dm in height. The plant is coarse, more or less appressed-hairy or sub-glabrous It has burs which are broadly cylindrical to ovoid or subglobose, about 1-3 5 cm long covered with stout hooked prickles, terminated by two straight or in some more or less incurved beaks. It is found in fields, waste places, flood plains, and lake and sea beaches. Now it is a cosmopolitan weed probably originally native to North America. It is a C_3 species.

1.3 Sand Accretion

Sand accretion could bury plants to varying depths and in some locations the amount of sand movement could be considerably high. For example, sand accretion along Lake Michigan was 30 cm per year (Olson, 1958a), 9 cm per year along Lake Huron (Maun, 1986) and 20 cm over two weeks at some locations along Lake Erie (Yanful, 1988) Sand movement could be in the form of deposition of sand (accretion), where the sand buries seeds, seedlings and adult plants to various depths, or the removal of sand, where the reverse occurs. These activities affect the life history of dune plants in a number of ways.

For instance, sand accretion may bury seeds to varying depths and affect the germination and emergence of the seedling. Maun and Lapierre (1986), and Zhang and Maun (1989) pointed out that germination of *Ammophila breviligulata* Fernald and *Agropyron psammophilum* Gillett & Senn seeds decreased with increasing depth of sand burial The reduction of seed germination at greater depth may be the result of poor aeration, temperature fluctuations, excessive moisture content, light quality and

periodicity (Harper, 1977; Zhang and Maun, 1990a, Fenner, 1985). Sand accretion also reduces seedling emergence in a number of species including *Ammophila breviligulata*, *Elymus canadensis* L., *Panicum virgatum* L., *Agropyron psammophilum*, *Cakile edentula* (Bigel.) Hook., and *Corispermum hyssopifolium* L. (Maun and Lapierre, 1986; Zhang and Maun, 1989, 1990a). Maun and Lapierre (1986) reported that the emergence of seedlings was primarily related to the amount of stored food reserves in the seed. The larger the seed, the greater is its capacity to emerge from sand burial.

Sand accretion also affects the establishment and growth of dune plants. For instance, sand burial over 6 cm killed one- and two-week old seedlings of *Agropyron psammophilum* (Zhang and Maun, 1990a). Seedlings of *Panicum virgatum* failed to withstand burial depths of only 1.6 cm when they were one-week old and 3.5 cm when two-week old (Zhang and Maun, 1990b).

According to Harris and Davy (1986), shoots of *Elymus farctus* L. usually could not withstand more than 15 cm of sand deposition. Shoot densities of adult plants of *Ammophila breviligulata* and *Calamovilfa longifolia* (Hook.) Scribn. were reduced by sand deposition exceeding 20 cm (Maun and Lapierre, 1984; Maun, 1985).

The fact that many dune plants have a strong ability to tolerate sand accretion is a well documented phenomenon. For example, a study in England showed that *Ammophila breviligulata* and *Elymus farctus* were able to withstand 90 cm and 20 cm of sand deposition, respectively (Ranwell, 1972). Plants buried by sand may also result in enhanced growth and lead to genetic differentiation in plant populations. Johnson (1978) reported an ecotype of *Abronia maritima*(Nutt.) in sand depositing habitats along the Pacific Coast, which grew 3.5 times faster than populations where there was no sand deposition. Morphological ecotypes of *Atriplex leucophylla* L. along the Pacific Coast may have evolved in response to differential rates of sand movement (de Jong, 1979). Seedlings of *Agropyron psammophilum* showed improved growth when buried up to 25 percent of their height (Zhang and Maun, 1989).

Growth stimulating effects of sand accretion on *Ammophila breviligulata* are very pronounced. For instance, the biomass of rhizomes, root fractions, and shoots increased significantly with increasing burial depth (Disraeli, 1984, Maun and Lapierre, 1984). Furthermore, it was shown that the absence of sand deposition could

drastically reduce the shoot biomass and flowering of *Ammophila breviligulata* (Eldred and Maun, 1982, Laing, 1954; Marshall, 1965; and Woodhouse, 1982).

In spite of the extent of research in this area relating to sand accretion and its effect on plant growth, there has been no conclusive explanation of this phenomenon. However, there are several explanations offered by the various researchers are as follows.

(i) Sand accretion may protect the root system of plants from desiccation (McLeod and Murphy, 1977). For example, the mid-day summer temperature of sand surface of Lake Michigan sand dunes could be 50 C or higher and at this temperature the top 10 cm of the sand may have less than half the possible moisture storage capacity (McLeod and Murphy, 1977). Thus, sand deposition can raise the sand surface and hence protect the plant's root system from desiccation

(ii) Sand accretion may provide a greater nutrient base and increase the rooting surface for plants Many studies have shown that sand dune environment is poor in nutrients such as nitrogen, phosphorus, and potassium (Baldwin and Maun, 1983; Hawke and Maun, 1988; Willis, 1963; Willis, 1985; and Gibson, 1988). Therefore, sand deposition may add to the potential nutrient reserves of the sand dune environment However, according to van der Putten (1989), the contribution of nutrients by the added sand is minimal. Furthermore, since the root systems of dune plants are largely found within the top 40 cm of the sand surface (Maun, 1985), sand accretion could develop a new zone for the initiation of adventitious roots on the accreted shoots (Olson, 1958b; Marshall, 1965).

(iii) Sand accretion may provide the active root zone a substratum free of pathogenic soil microorganisms. Studies conducted by van der Putten (1989) suggested that newly accreted sand (mobile sand) is usually free of pathogenic microorganisms and van der Goes (pers comm.) suggested that soil microorganisms such as nematodes take a longer time to infect the new roots than do mycorrhizal hyphae.

(iv) Sand accretion may increase symbiotic nitrogen-fixation and mycorrhizal association in the root zone, thus increasing the nutrient uptake and growth of plants (Morris *et al.*, 1974).

(v) The deposition of sand may reduce plant density by eliminating plants

intolerant to sand accretion and thus reducing interspecific competition (Huiskes, 1979, Disraeli, 1984).

(vi) Sand accretion increases net photosynthetic rate, thereby increasing the metabolic requirements of plants. Studies done by Yuan *et al.*, (1993) showed that the net photosynthetic rate was increased in *Ammophila breviligulata* and *Calamovilfa longifolia*.

Numerous studies have been done to determine the effect of sand accretion on dune plants, but there are very few studies which examine the physiological aspects of plant response to sand accretion, the community aspects of dune ecology, and the role of mycorrhizae in sand dunes

This thesis examines physiological and morphological responses of several annual, biennial and perennial dune species The studies include three aspects of plant growth-sand burial relationship.

I The plant community distribution in the sand dunes along Lake Erie and Lake Huron and its relation to sand accretion.

II. Investigations of CO_2 uptake to determine the photosynthetic capacity by infrared gas analysis and the photochemical efficiency (F_v/F_m) using the LICOR-6200 CO_2 gas analyzer and plant stress meter respectively.

III. A survey of the extent of mycorrhizal colonization in dune species has been done and the effect of mycorrhizae on the growth of buried plants

1.4 Thesis Objectives

(i) To study the effects of burial on changes in composition of plant communities, in natural sand depositing sites and in artificially buried sites, along Lakes Huron and Erie.

(ii) To examine the effect of burial to various depths, on the ecological, physiological, and morphological traits of several common dune species

(iii) To determine the extent of vesicular-arbuscular mycorrhizal (VAM) colonization among dune plants along Lakes Erie and Huron and the effects of VAM

on plant growth under burial conditions

1.5 Study Site

1.5.1 Pinery Provincial Park

The Pinery Provincial Park situated in Lambton County, Ontario, Canada (43 15'N, 81 50'W) (Fig. 1 1) was one of the two sites chosen to conduct the field studies for this project. The climate of this area is temperate and has a mean monthly temperature range of 13 to 21 C in summer and 0 to -6 C in winter. Monthly mean precipitation ranges between 7 and 10 cm with a yearly average of about 90 cm (Baldwin, 1983). The sand dunes range in height from 6 to 13 m above the present lake level and represent a chronosequence dating 5000 years B.P (Morrison and Yarranton, 1974, Baldwin and Maun, 1983).

The study was done on a 1 km section of the first dune ridge in an area of the Pinery called "Wilderness Area" (Fig. 1.2). This site was chosen because it is relatively inacessible to park visitors, reached only on foot. A fenced plot located at Picnic area 9 of the Day-use areas was also used for studies involving the artificial burial of plant communities

Micro-meteorological measurements recorded under still, cloudless conditions indicated a high diurnal soil temperature of 26 C at the soil surface layer and a more constant temperature at greater depths. The maximum diurnal air temperature was highest at the soil surface (around 33 C) and decreased with height above the soil surface (160 cm) to 23 C (Chang, 1968; Baldwin and Maun, 1983). The photosynthetically active radiation (PAR) readings in the slack (low region between first and second dune ridges) at 1400 h was 1800 μmol m^{-2} s^{-1} (Baldwin and Maun, 1983) In general the soil in the study area on the first dune ridge of the Pinery Provincial Park was characterised by low organic matter, low water retention capacity, and fine sand texture

Figure 1.1 Map of southwestern Ontario showing the location of Pinery Provincial Park and Port Burwell Provincial Park.

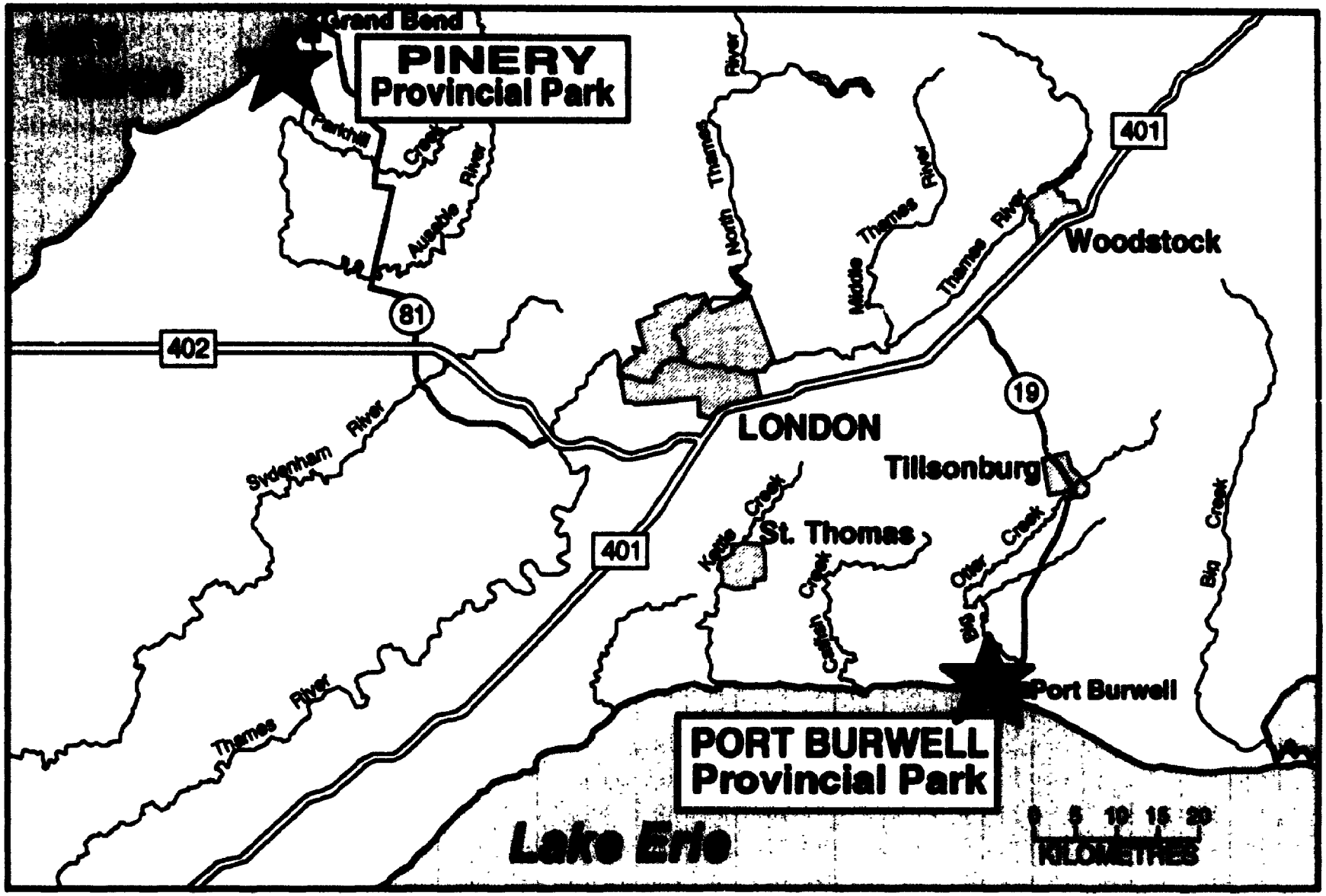
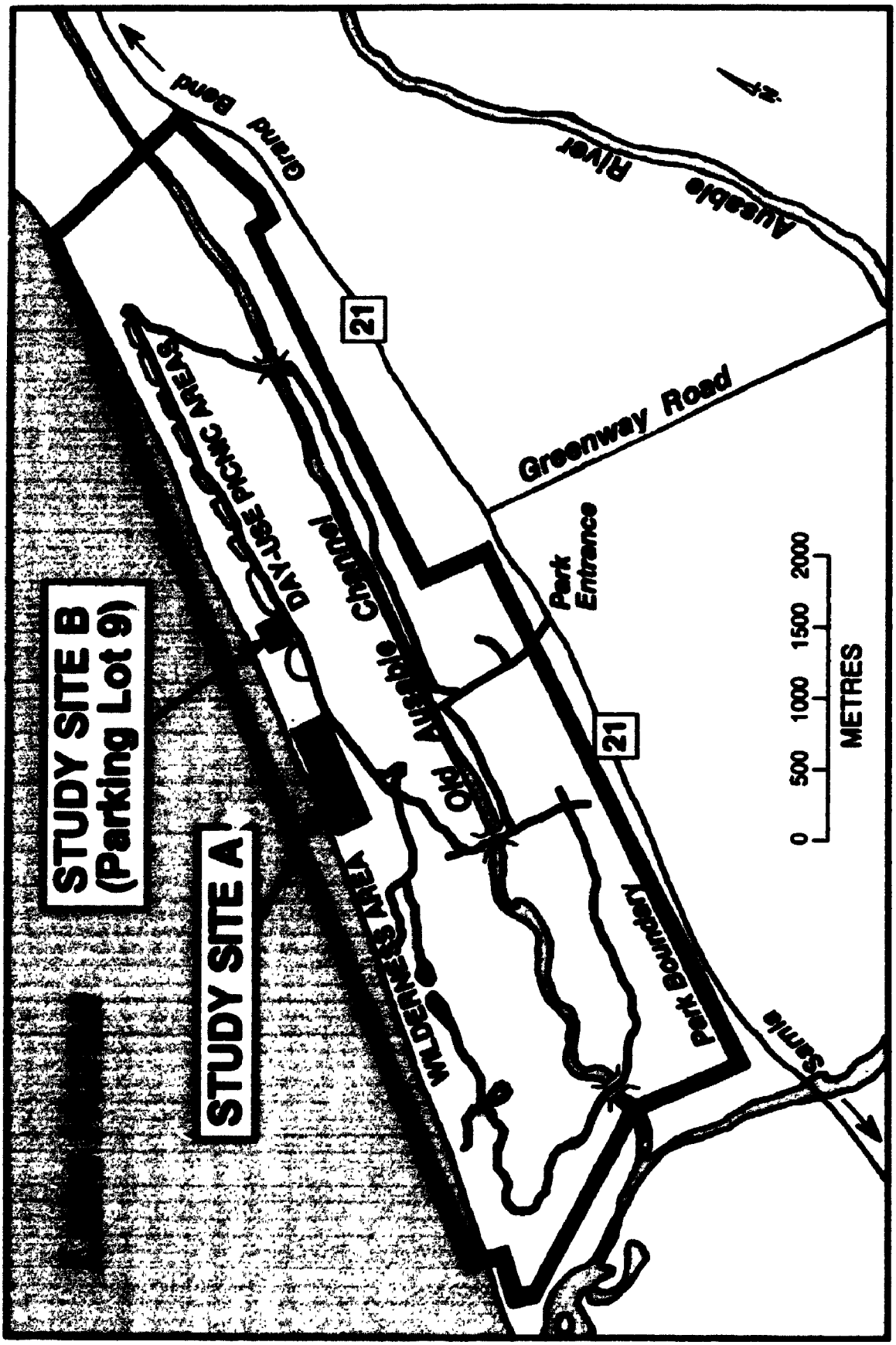

Grand Bend
PINERY
Provincial Park
Franklin
Creek
Ausable River
North Thames River
Middle Thames River
Thames River
401
Woodstock
81
402
19
LONDON
Tillsonburg
Sydenham River
Kettle Creek
St. Thomas
401
Otter Creek
Big Creek
Catfish Creek
Port Burwell
Thames River
PORT BURWELL
Provincial Park
Lake Erie
0 5 10 15 20
KILOMETRES

Figure 1.2 Map of the Pinery Provincial Park showing the location of the study site A (Wilderness Area) and study site B (Picnic Area 9)

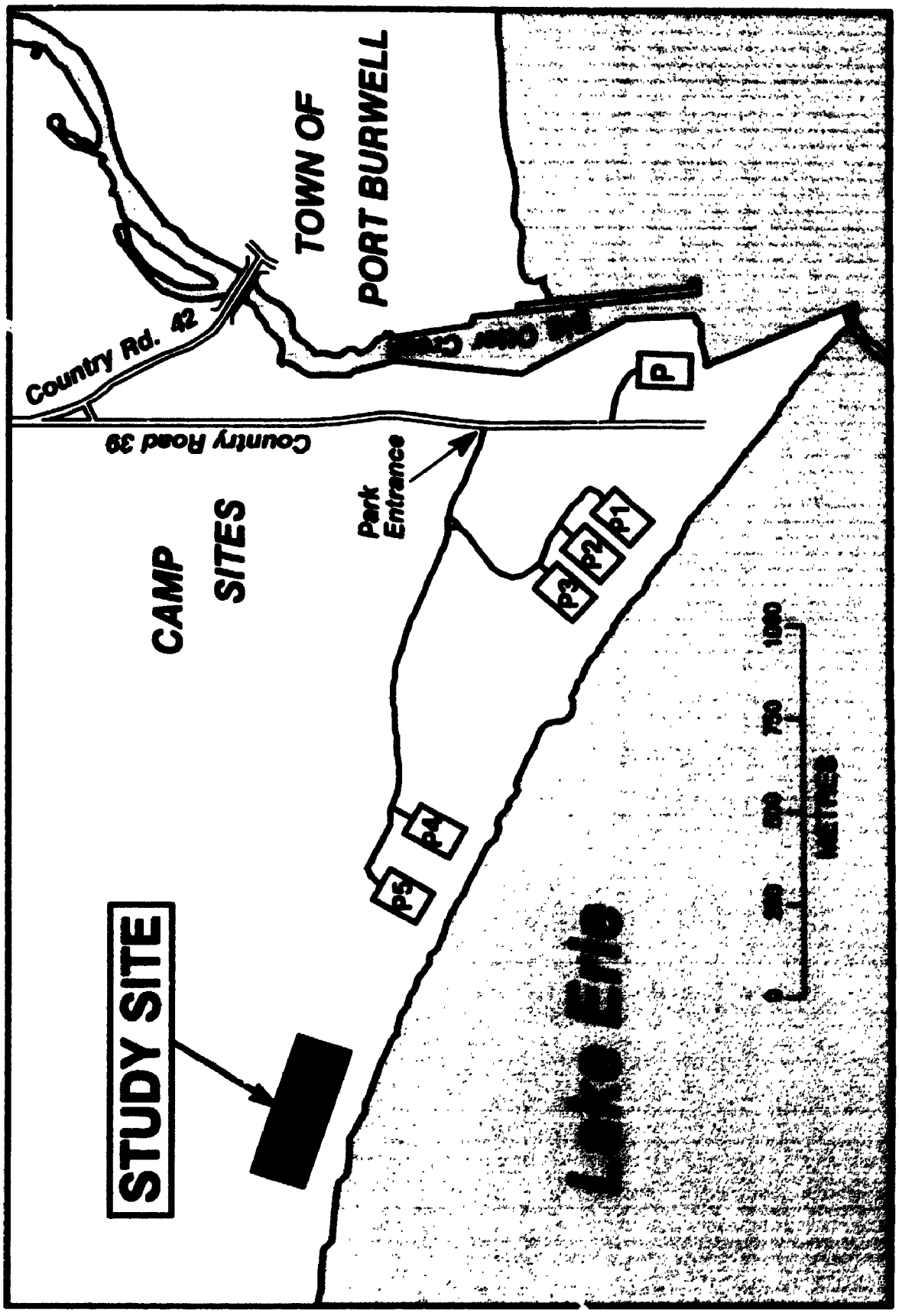

STUDY SITE B
(Parking Lot 9)
STUDY SITE A
DAY-USE PICNIC AREAS
Grand Bend
21
Greenway Road
Park Entrance
Old Ausable Channel
WILDERNESS TRAIL
Ausable River
Park Boundary
Lambton
0 500 1000 1500 2000
METRES

1.5.2 Port Burwell Provincial Park

The second study area was at the beach along Lake Erie at Port Burwell (Iroquois Beach) Provincial Park (42 40'N, 80 51'W) This park has an area of 640 acres and is located adjacent to the fishing village of Port Burwell at the mouth of Big Otter Creek, Elgin County, Ontario, Canada The "foredune" at the Port Burwell Provincial Park is a low-relief zone composed of an indistinct series of beach ridges (Yanful, 1988). Location of study site was on the beach in the northwestern end of the park (Fig 1.3). The area is subjected to flooding and overwash during storms and large amounts of driftwood, tires, and detritus are deposited on the drift line (Yanful, 1988). This region generally experiences a modified climate due to the influence of the surrounding Great Lakes (Klinkenberg, 1984) The micro-climatic factors vary in the study area The micro-climate on the south-facing bluffs and beach plains are warmer than the plateau area (Klinkenberg, 1984)

The sandy beach plain area of the park is formed since 1923 as a result of the building of a pier which altered the lake currents in the area, and thus trapped the eastward movement of sediments (Klinkenberg, 1984). The beach formation may be the result of subsequent additional sand deposition on the sand bars that already existed at the mouth of the Big Otter Creek, which runs parallel to the shoreline (A Maun, pers comm)

Figure 1.3 Map of Port Burwell Provincial Park showing the location of the study site on the west of picnic area 5


TOWN OF
PORT BURWELL
Country Rd. 42
Country Road 39
Park Entrance
CAMP
SITES
STUDY SITE
P
P1
P2
P3
P4
P5

CHAPTER TWO

SAND MOVEMENT AND SPECIES DISTRIBUTION

2.1 Introduction

Mobility of sand is a recurrent event in sand dune systems Its stability is determined by vegetation type, rate and amount of sand accretion, and the ability of vegetation to withstand the burial stress According to Moreno-Casasola (1985) there is a close interaction among plant species, vegetation cover, soil movement and dune shape Sykes and Wilson (1990) suggested that a complex of environmental factors affects species distribution on dunes, among which sand burial is one of the most important The mobile sand buries seeds, seedlings, and adult plants to various depths and determines the plant community composition and structure of the dunes. Sand movement affects seedling establishment and adult populations in different ways (Barbour *et al.*, 1984, Holton and Johnson, 1979) It is probably one of the important factors that contributes to the formation of mosaic patterns of vegetation in the dunes. A high rate of sand mobility inhibits the growth of any but highly specialized plant species (Salisbury, 1952).

A large micro-climatic variation is detectable in different parts of a dune system according to local shelter effects, proximity of the ground water table and distance from the lake This is reflected in complex mosaic patterns of vegetation (Ranwell, 1972) The role of wind and consequently of sand movement in dunes has been reported for a long time (Moreno-Casasola, 1986; Cowles, 1899; Ranwell, 1972 and Salisbury, 1952) Nevertheless, there are few studies that quantified the relationship between sand movement and the occurrence of dune species. The effects of sand deposits on plants along Lakes Huron and Erie dunes have been studied primarily on single species but no studies have examined the distribution of species in the sand dune community The objective of the present study, is to better understand the effects of sand movement on spatial distribution of plant species in Lakes Erie and Huron sand dune systems

2.2 Materials and Methods

2.2.1 Natural sand accretion and species distribution

The area selected for field observations was located on the sand dunes along the south-eastern shore of Lake Huron near Grand Bend, Ontario The study site was chosen in the "Wilderness Area" of the Pinery Provincial Park in May, 1992. The objective was to record the amount of sand movement and the corresponding occurrence and establishment of plant species An area approximately 600 m long, parallel to the shoreline and 50 m wide perpendicular to the shoreline, with little or no disturbance by picnickers, was staked for this study (Plate 2.1)

The amount of sand accretion was measured by installing 96 steel stakes (2 m long and 6 mm in diameter) which had been painted white to avoid vandalism during winter The stakes were buried to a depth of 120 cm at regular distances (approximately 3 m apart) along 24 transects (approximately 5 m apart) perpendicular to the shoreline, from the windward 'ge of the first dune ridge beginning with the crest, followed by the upper slope, the lower slope, the leeward slope and ending with the slack (Plate 2.1a). The amount of sand accumulated (+) or eroded (-) at each stake was recorded once a month for two years, except in the winter months when the dunes were covered with snow

The abundance of plant species was recorded by placing a one meter quadrat on the ground with the steel stake at the centre of the quadrat and one side of the quadrat parallel to the shoreline of the lake This ensured that the same area was monitored at each count over the period of study Species count and percentage cover were recorded in each quadrat using the Braun Blanquet method (Westhoff and van der Maarel, 1978)

Plate 2.1 a The study site in the "Wilderness Area" at the Pinery Provincial Park where the stakes were installed on the crest (c), upper slope (u), and lower slope (l) of the first dune ridge and in the slack (s).

b One of the 96 stakes installed at the study site to monitor sand movement


a


b

2.2.2 Experiment I: Effect of artificial burial in sand on survival, growth and relative abundance of plant species at pinery Provincial Park along Lake Huron

In May, 1992 an area approximately 15 x 25 m was fenced at the beach of Pinery Provincial Park to determine the effects of experimental sand accretion on distribution, survival and relative abundance of plant species (Plates 2.2 & 2.3). The area was divided into thirty-six squares and one wooden frame (1 x 1 x 0.2 m deep) was placed over plants in each of the thirty-six squares Percent cover of all the plant species occurred in the wooden frames were recorded The burial treatments with sand were then applied inside the frames. Treatments consisted of O (control), 5, 10, 20, 40, and 80 cm of burial with six replications per treatment For 40 and 80 cm burial depths 2 or 4 frames were stacked and then filled with sand. The 36 treatment combinations were completely randomized in the 36 squares as shown in Fig. 2.1. Changes in presence and coverage of species were recorded once a month for two years using the modified Braun Blanquet method (Westhoff and van der Maarel, 1978)

The experiment was terminated between August 18-24, 1993, after about 15 months First, all stems of emerged plants in the frames were marked with a permanent marker at the soil level so that following harvest the mode of growth and regeneration of each species could be studied below the soil surface. At the end of the treatment period the wooden frames were carefully lifted and the sand around each stem was manually removed to a depth of about 30 cm below the preburial soil surface At this point the plants were dug out of the sand using a shovel. Rough sketches of the morphological growth pattern of different species following burial were drawn to determine the mode of emergence from the burial treatment. The plants were then placed in marked paper bags and taken to the laboratory, where they were kept in a cool room at 4°C until further measurements

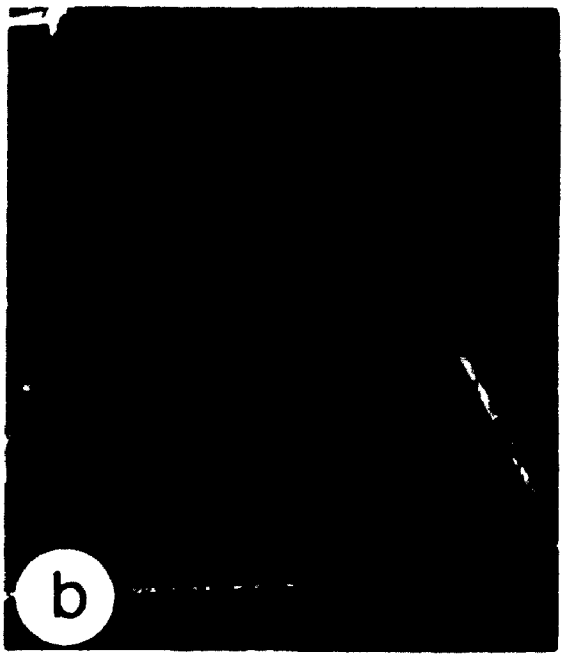
Four culms or stems of each species were chosen randomly from each replication, except where less than four stems were present in a box, as was often the case in the 40 and 80 cm burial treatments. The plants from the sample were used for

Plate 2.2 a The study site in fenced area enclosing Experiment I behind the beach at Pinery Provincial Park along Lake Huron

b Control treatment (0 burial) of Experiment I showing in the foreground

c The 10 cm burial treatment of Experiment I 14 months following the treatment


a


b

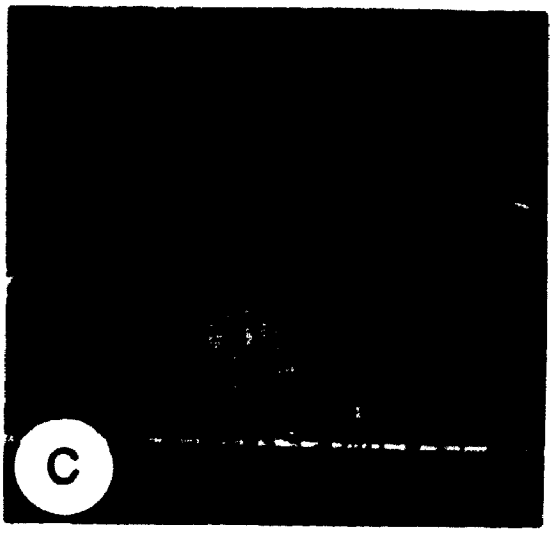
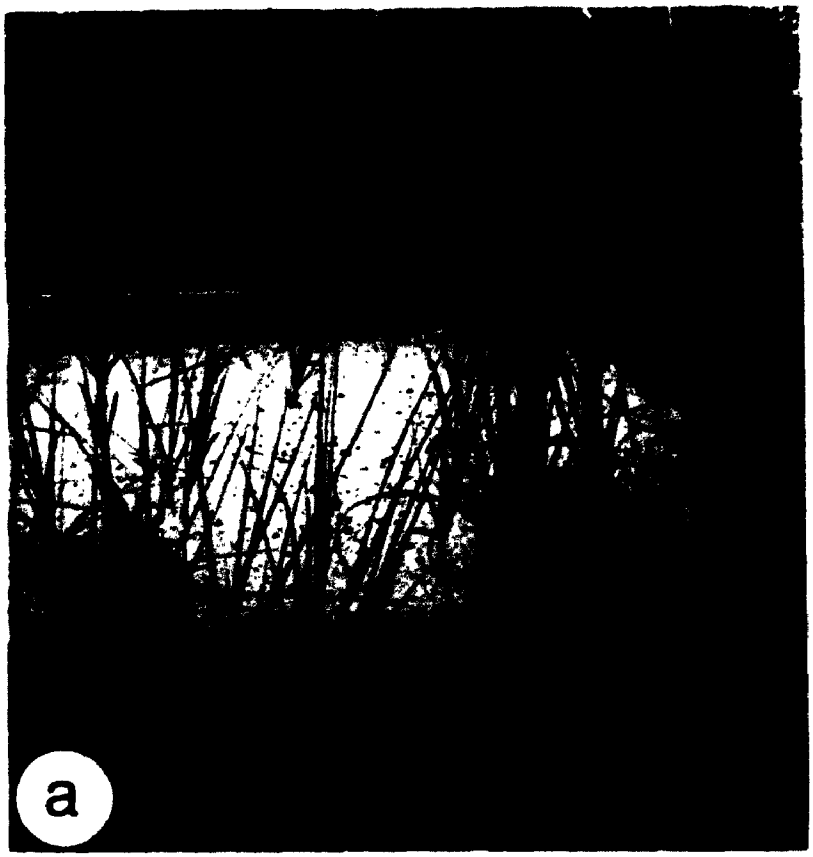
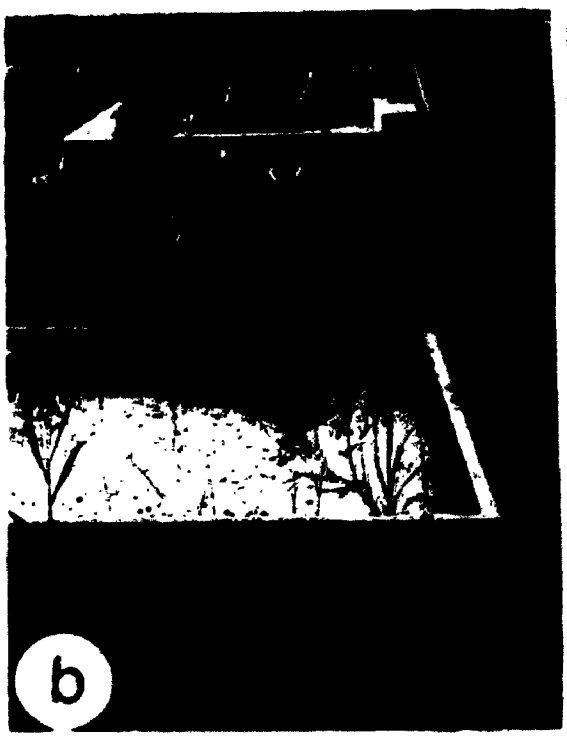

c

Plate 2.3 Some burial treatments of Experiment I, (a) 20 cm, (b) 40 cm and (c) 80 cm at Pinery Provincial Park along Lake Huron


a


b

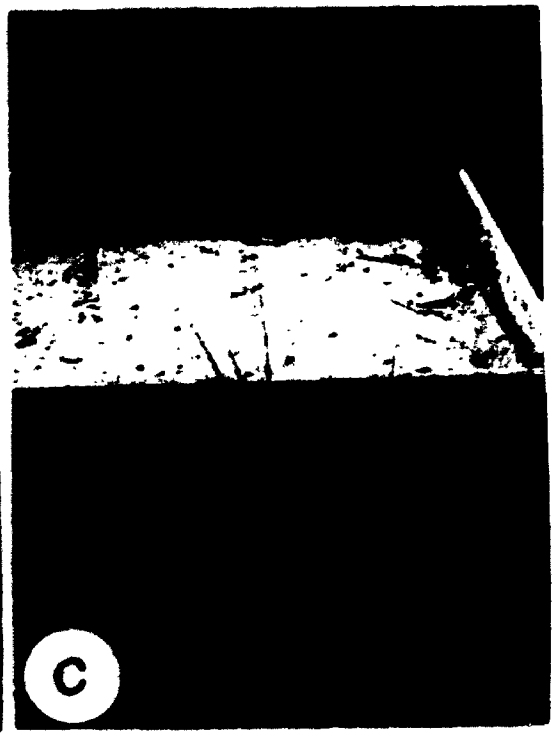
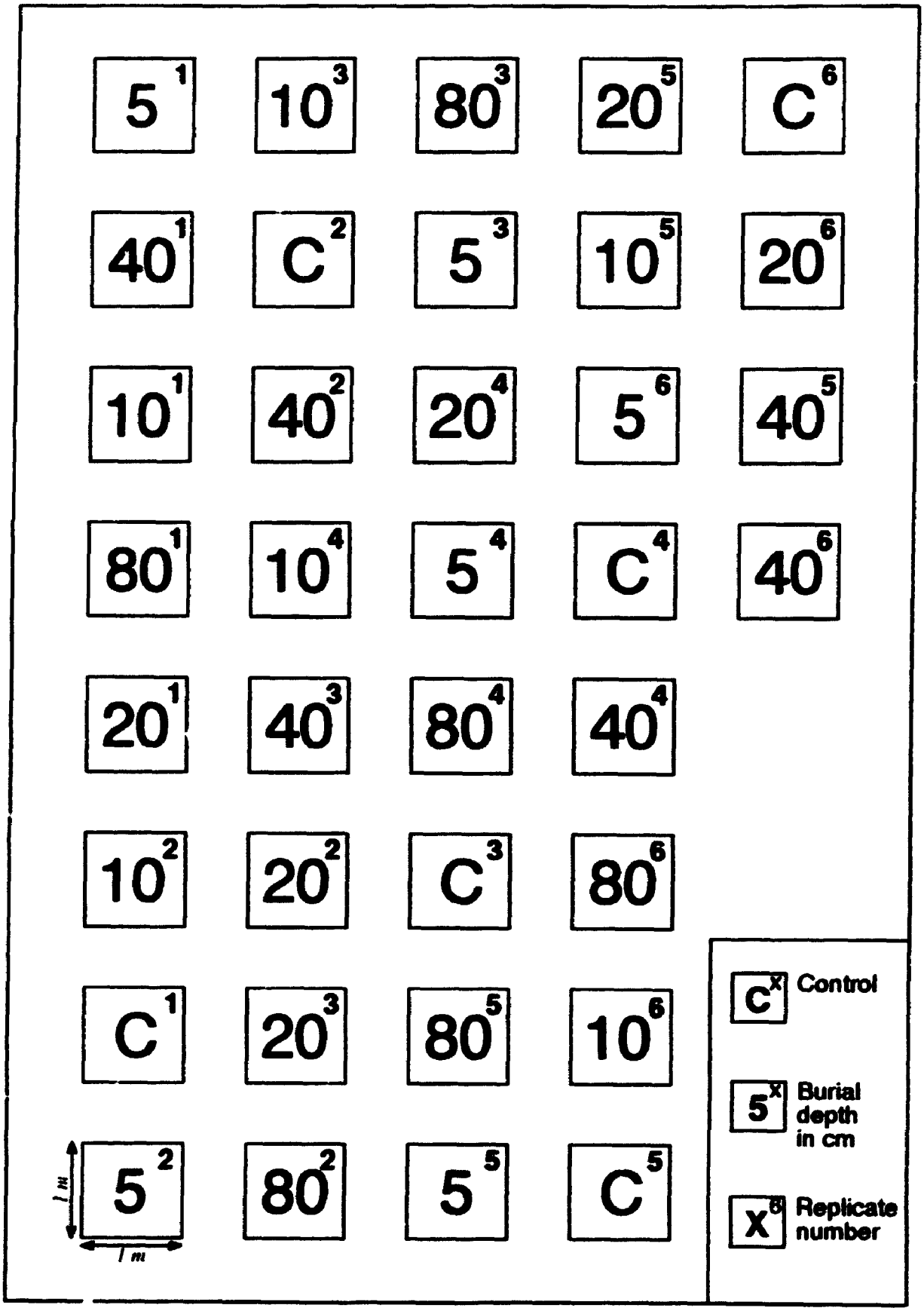

c

Figure 2.1 Layout of completely randomized treatments in the study site for Experiment I. The burial depth is recorded in block letters while replication number is in the top right hand corner of each square.


5 1
10 3
80 3
20 5
C 6
40 1
C 2
5 3
10 5
20 6
10 1
40 2
20 4
5 6
40 5
80 1
10 4
5 4
C 4
40 6
20 1
40 3
80 4
40 4
10 2
20 2
C 3
80 6
C 1
20 3
80 5
10 6
5 2
80 2
5 5
C 5
1 m
1 m
C X Control
5 X Burial depth in cm
X R Replicate number

recording height, number of nodes per culm or stem, length of internodes, biomass per frame and root/shoot ratio

Dry biomass of each species for each replication was recorded by including all parts of excavated plants to a depth of about 30 cm below the preburial surface The ratio of below-ground (roots + rhizomes) to above ground biomass was then calculated

2.2.3 Experiment II: Effects of artificial burial on survival, growth and relative abundance of plant species at Port Burwell Provincial Park along Lake Erie

Most of the plants used in Experiment I were grasses, therefore an identical experiment was laid out at Port Burwell Provincial Park along Lake Erie (Plate 2 4) in which annuals and biennials were included Burial technique was identical to Experiment I, with the exception of the size of the wooden frames, which were smaller (0.5 x 0.5 x 0 2 m deep) Four burial treatments, 0 (control), 5, 10, and 20 cm of sand were superimposed on plants and ten replicates were used (Fig 2 2) Species present and percent cover of each species were taken once a month for two years beginning in May, 1992, except during the winter months The experiment was terminated from August 25 to August 30, 1993, following procedures similar to those of Experiment I

2.2.4 Statistical Analysis

Data collected for the relationship between sand accretion and species distribution (section 2 2.1) were first analyzed by using a multivariate analysis of variance (MANOVA) program called EMVO (Ecological program for instructional computing on the Macintosh, by László Orlóci, 1991), to see if the length of time following burial significantly contributed to variation in species abundance The results showed that there was no significant difference, hence, data from all the months were pooled. An ordination was then done using CONAPACK, a programme for Canonical Analysis of classification tables (Feoli and Orlóci, 1979) to establish a gradient in the sand movement and the distribution of species Furthermore a PCA program PCAR

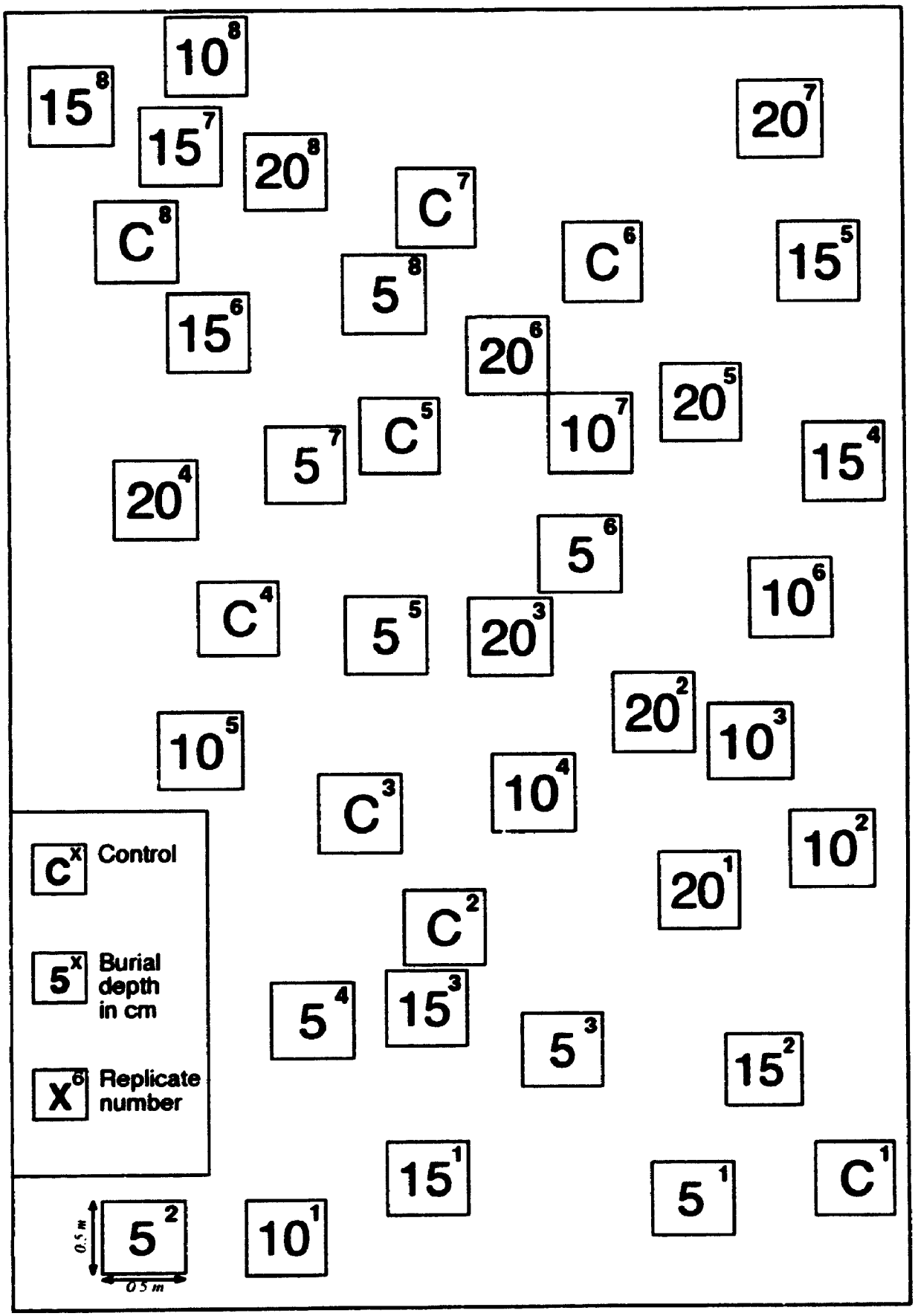
Plate 2.4 a Study site at the beach of Port Burwell Provincial Park along Lake Erie

b A replication of 20 cm burial treatment


a


b

Figure 2.2 Layout of completely randomized treatments for Experiment II. The burial depth is recorded in block letters while replication number is given in the top right hand corner of each square.


10 8
15 8
15 7
20 8
20 7
C 7
C 8
C 6
15 5
5 8
15 6
20 6
20 5
10 7
C 5
5 7
15 4
20 4
5 6
C 4
10 6
5 5
20 3
20 2
10 3
10 5
C 3
10 4
10 2
20 1
C 2
5 4
15 3
5 3
15 2
15 1
5 1
C 1
5 2
10 1
0.5 m
0.5 m
C X Control
5 X Burial depth in cm
X 6 Replicate number

(Orlóci, 1991) was plotted using the square root of eigenvalues to determine the association between the species since *Arctostaphylos uva-ursi*, *Cakile edentula*, *Corispermum hyssopifolium* and *Equisetum arvense* were present in only 2 or 3 quadrats they were not included in further analysis.

The differences between treatments in Experiments I and II were determined by one way analysis of variance (ANOVA) followed by Tukey's Test to determine if the means were significantly different (Zar, 1984). Bartlett's test was done to test the equality of the variances and where the data did not meet the assumption, the data were log transformed. Canonical Group Analysis from the programs EMVO and PLOT was performed to analyze the data recorded for both Experiments I and II.

2.3 Results

2.3.1 Natural Sand Accretion and species distribution

The results from EMVO indicated that there was no association between "time" and "species abundance" The chi-square value from the canonical correlation analysis (Table 2 1) was significant 334 3 $P<0.05$, d f=130. Therefore, the null hypothesis that there was no association between species and sand movement, was rejected, and alternate hypothesis that there was an association between species distribution and sand movement was accepted

The following 11 species were found at the study sites, *Ammophila breviligulata*, *Andropogon scoparius*, *Artemisia campestris*, *Arctostaphylos uva-ursi*, *Cakile edentula*, *Calamovilfa longifolia*, *Corispermum hyssopifolium*, *Equisetum arvense*, *Juniperus communis*, *Lithospermum caroliniense*, and *Populus balsamifera*.

The lower part of Table 2 2 shows the absolute change in the amount of sand movement regardless of the direction of change at each stake over the study period. Microsites which had erosion are indicated with a -, and those with sand accumulation with a + In the microsites where both erosion and accumulation had occurred, both - and + signs were used In this instance the process (erosion or accretion) which accounted for greater amount of sand movement was indicated first (Table 2.2). The

Table 2.1: Canonical correlations and cumulative percentages of data explained by each partition.

Partition	Canonical correlations (r)	Cumulative percentages
1	0.60	31.90
2	0.52	55.62
3	0.38	68.44
4	0.36	79.91
5	0.31	88.31
6	0.26	94.32
7	0.20	97.84
8	0.15	99.84
9	0.04	100.00

$\chi^2 = 334.27 > \chi^2\ 0.05, 130\ (157.5)$
Null hypothesis that sand movement and species distribution are independent is rejected. Therefore, species distribution is dependent on sand accretion.

Table 2.2: Percent coverage and sand movement of 94 releves grouped according to the topography of the dune, (+) indicating sand accretion and (–) indicating erosion.

Group	1 (crests)	2 (upper slope)	3 (lower slope)	4 (slack)
Releve number	000111122233444446777888990 235127603468046793789238 12	000111112222334455555566666677778 9 789345695678041223567901456901355 0	555778888889999 018240146793456	001222333333444566 7 460129123579358427 6
1 *Ammophila breviligulata*	--7----------6---65-76---	--7-7----6----6----------	----------	----------
2 *Andropogon scoparius*	---5---6--55-756-7--5---67	7----665--6-5--6-----7567------766	--5-57566676666	--576-57-556557----
3 *Artemisia campestris*	----------5---5-------	----------5----------	------5-------	------5-5---------
4 *Calamovilfa longifolia*	8566-6858-7-8-7776666-656-	5-565557767577868-6576665677577556	78668--5-5---5-	55776755----65--56-
5 *Juniperus communis*	-6-69----9----------	-75----------9---------8-----	----------	6------6-------9--9
6 *Lithospermum carolinese*	-------5----------	-----555----5--------5--5-----5---	----------55--	-5------55-5-------
7 *Populus balsamifera*	-----6--5---5----------5-	----------5----------55	----------	----------
Sand movement (cm)	45420545023453424435573353 836950911324213997181418 21	043203340232213234233333112021103 0 883833622315158859227975979103536 2	012010000001110 030121045161046	000000000000000000 030000031430320262 1
Erosion and/or accretion	++++-++++++++-++++++++++-- --	-++++----------+---+- + ++- -+++-+-- + + + - -- - - ----		

sites with the greatest amounts of sand movement were generally the crests, followed by the upper slope The lower slopes and the slack region had relatively low sand movement (Fig 2 3)

The PCA ordination shows the distribution of releves in relation to the first two axes (Fig 2 4) The subsequent axes provide little information The eigenvalue for the first vector was 10 15 or 30 82% (cumulative 30 82%) and the eigenvalue for the second vector was 9 66 or 29 33% (cumulative 60 15%) The relevé ordination shows a species gradient which can be correlated with the topography of the dunes and the amount of sand movement in each region of the dunes *Arctostaphylos uva-ursi*, *Cakile edentula*, *Corispermum hyssopifolium* and *Equisetum arvense* were not included in the analysis because they were present in only 2 or 3 sites

The species groups described in Table 2 2 were superimposed on the ordination graph (Fig 2 4) Their distribution was correlated with the amount of sand movement as shown in figure 2 3 The crest (group 1) with *Ammophila breviligulata*, *Andropogon scoparius*, *Artemisia campestris*, *Calamovilfa longifolia*, *Juniperus communis* and *Populus balsamifera* occurs on the left side of the graph (Fig 2 4) in regions of greater sand movement The releves of the upper slope (group 2) in which all the species of group 1 were present with the addition of *Lithospermum caroliniense* appearing on the lower right side of Fig 2.4. The lower slope (group 3) where *Ammophila breviligulata*, *Juniperus communis* and *Populus balsamifera* were absent, appear on the upper right side of the ordination graph (Fig 2 4) In the slack region (group 4) where only *Ammophila breviligulata* and *Juniperus communis* were absent the ordination appeared on the lower part of the graph (Table 2.2 and Fig 2 4)

The distribution of the seven dune species was analyzed by means of an average linkage cluster analysis, UPGMA (unweighted pair-group method using arithmetic averages) The dendrogram derived from the analysis showed three main groupings the first group included *Ammophila breviligulata*, *Artemisia campestris*, *Populus balsamifera* and *Lithospermum caroliniense* (these species were present on the crests of the dunes). Juniperus communis (group 2) and *Andropogon scoparius (group 3)*, were more abundant on the south slopes and the slack; and the fourth group was *Calamovilfa longifolia* which was abundant on the crests and slopes but had reduced

Figure 2.3 Ordination of 94 relevés in which the absolute sand movement (cm) at each stake was recorded during the two years

Group 1 ▲ Crests

2 ● Upper Slope

3 ★ Lower Slope

4 ■ Slack

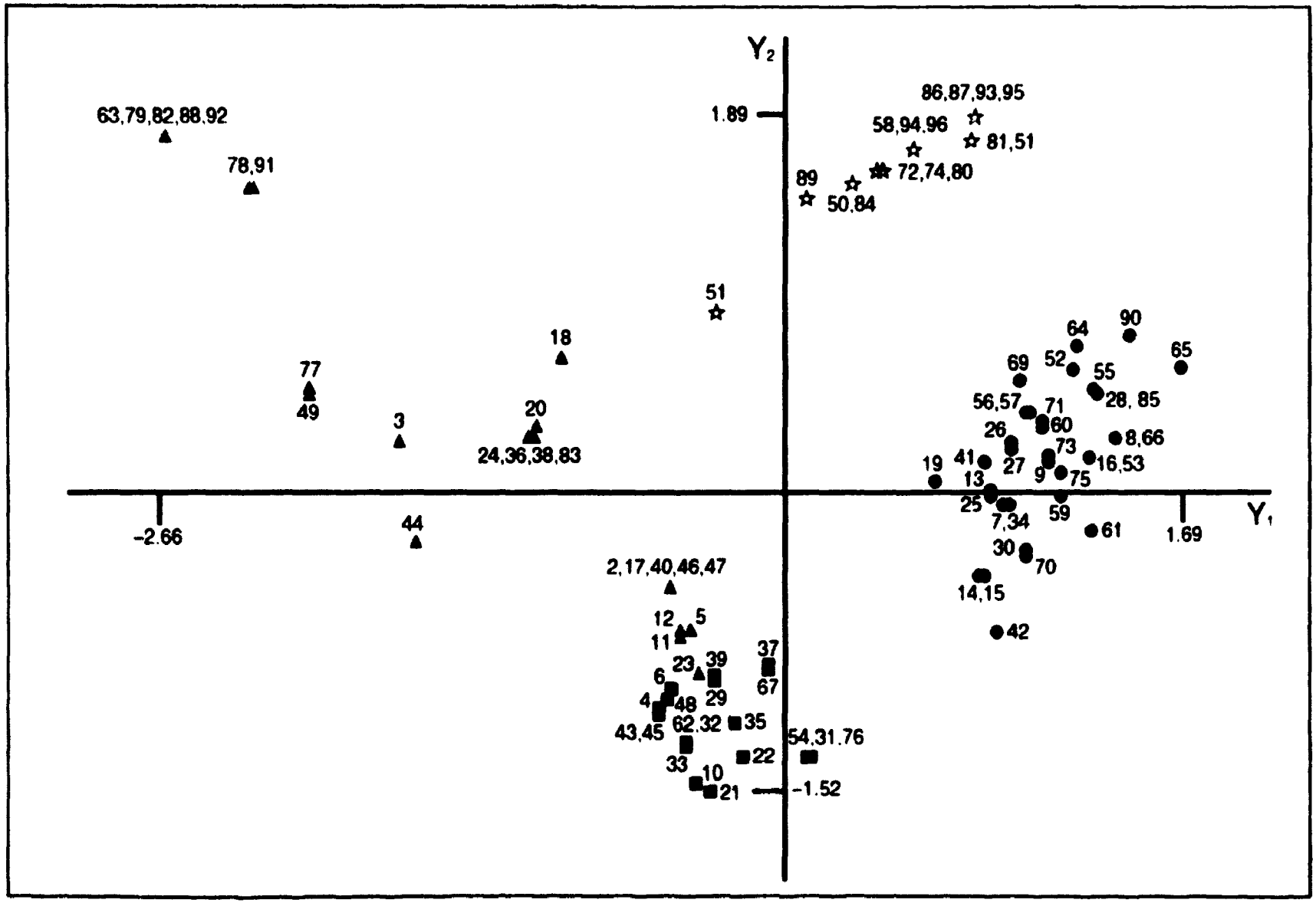

Y2
Y1
1.89
-1.52
-2.66
1.69
63,79,82,88,92
78,91
77
49
3
44
18
20
24,36,38,83
51
86,87,93,95
58,94,96
81,51
72,74,80
89
50,84
64
90
65
69
52
55
56,57
71
28, 85
26
60
8,66
73
19
41
27
9
16,53
13
75
25
7,34
59
61
30
70
14,15
42
2,17,40,46,47
12
5
11
37
23
39
67
6
4
48
29
43,45
62,32
35
54,31,76
33
22
10
21

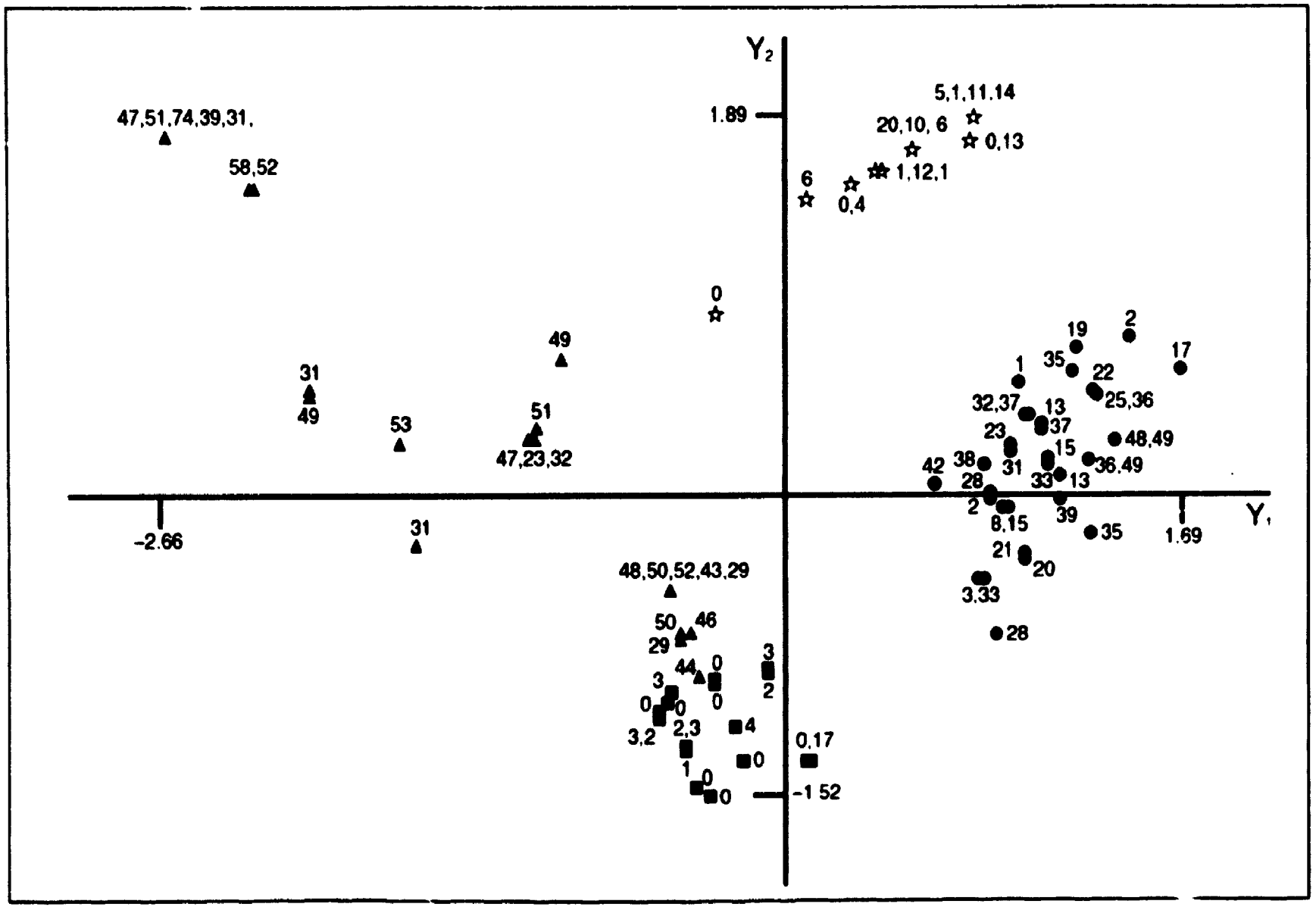
Figure 2.4 Scatter diagram showing the joint distribution of species in relation to the first two axes. The topographic groups obtained from Table 2.2 were superimposed on the ordination graph

Group 1 ▲ Crests

2 ● Upper Slope

3 ★ Lower Slope

4 ■ Slack


Y2
1.89
47,51,74,39,31,
58,52
5,1,11.14
20,10, 6
0,13
1,12,1
6
0,4
0
49
31
49
53
51
47,23,32
19
2
17
1
35
22
32,37
13
25,36
23
37
48,49
15
38
42
31
36,49
28
33
13
2
8,15
39
35
1.69
Y1
-2.66
31
21
20
3,33
28
48,50,52,43,29
50
46
29
44
0
3
2
3
0
0
0
2,3
4
3,2
0,17
1
0
0
0
-1 52

coverage in the slack region (Fig 2.5)

2.3.2 Experiment I. Effects of artificial sand burial on species abundance

For the analysis of data from this experiment it was assumed that the plant species in the community were randomly distributed prior to the imposition of the burial treatment. A multivariate program called CONAPACK was used to test if species abundance was associated with species type at the pre-burial stage. The calculated chi square value was 38.4 and the critical table value was 43.8. The null hypothesis (H_o) was therefore accepted, recognizing that there was no association between species type and species abundance. It was consequently accepted that the plants at the experimental site were randomly distributed before the imposition of burial treatments (Table 2.3)

Similarly, the same statistical technique (CONAPACK) was then used to test the association between the different sand burial treatments and species abundance using the final post-burial coverage data of each species. The calculated chi square value was 63.5 and the critical table value was 43.8. Thus, the H_o was rejected and it was established that there was an association between the species abundance and burial depth (Table 2.3)

Generally buried plants had greater amounts of root tissue and increased total plant height (Fig 2.6) *Ammophila breviligulata* exhibited an increase in its percent cover with the imposition of burial treatments. There was no significant increase in the cover of the control (0 cm) over time, but there were increases in the percent cover in the 5, 10, 20, and 40 cm burial treatments (Fig. 2.7). In these treatments the plants were not completely covered. They recovered quickly and started to show enhanced growth within the first month and soon had a cover of almost twice that of control plots. Only in the 80 cm burial treatment was the recovery slow and *Ammophila breviligulata* plants did not emerge until May, 1993, 13 months after burial. However, the new shoots showed a rapid increase in the percent cover soon after emergence (Fig 2.7)

Figure 2.5 Dendrogram for species distribution in the "Wilderness Area" of Pinery Provincial Park. The clustering method used was an average linkage (UPGMA). The numbers indicate relative units of distance at which group fusions took place.

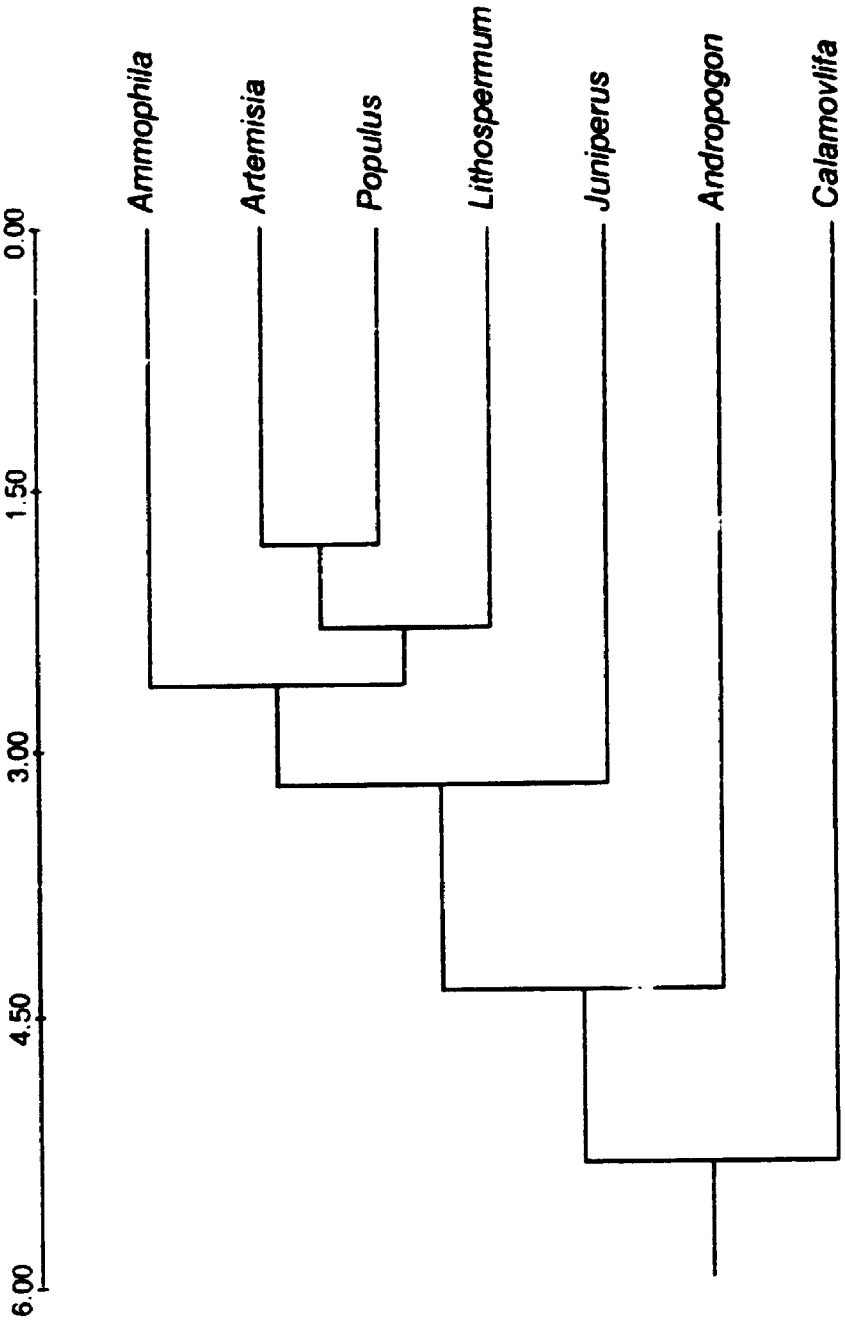

Ammophila
Artemisia
Populus
Lithospermum
Juniperus
Andropogon
Calamovilfa
0.00
1.50
3.00
4.50
6.00

Table 2.3: Canonical correlations and cumulative percentages of data explained by each partition.

Treatments	Partition	Canonical Correlations	Cumulative Percentages
Pre-burial	1	0.35	52.07
	2	0.28	85.40
	3	0.17	97.62
	4	0.08	100.00
	$\chi^2 = 38.36 < \chi^2\ 0.05, 30\ (43.77)$ Null hypothesis that species distribution and abundance are independent is accepted.		
Post-burial	1	0.48	56.92
	2	0.34	86.16
	3	0.21	97.64
	4	0.09	99.84
	5	0.03	100.00
	$\chi^2 = 63.54 > \chi^2\ 0.05, 30\ (43.77)$ Null hypothesis that burial depth and species distribution are independent is rejected. Therefore, species distribution is dependent on burial depth.		

Figure 2.6 Schematic drawings of six dune plants showing morphology of roots and/or rhizome at harvest. The original pre-burial surface for each plant is shown by the solid line close to its base. The broken line indicates the post-burial level of sand for the buried plants. Thus, the buried part of the plant is the distance between the solid and broken line in each drawing. Control and buried treatments of *Ammophila breviligulata* (a,b,c), *Andropogon scoparius* (d,e,f); *Calamovilfa longifolia* (g,h,i); *Elymus canadensis* (j,k,l), *Poa compressa* (m,n,o); *Lithospermum carolinense* (p,q)

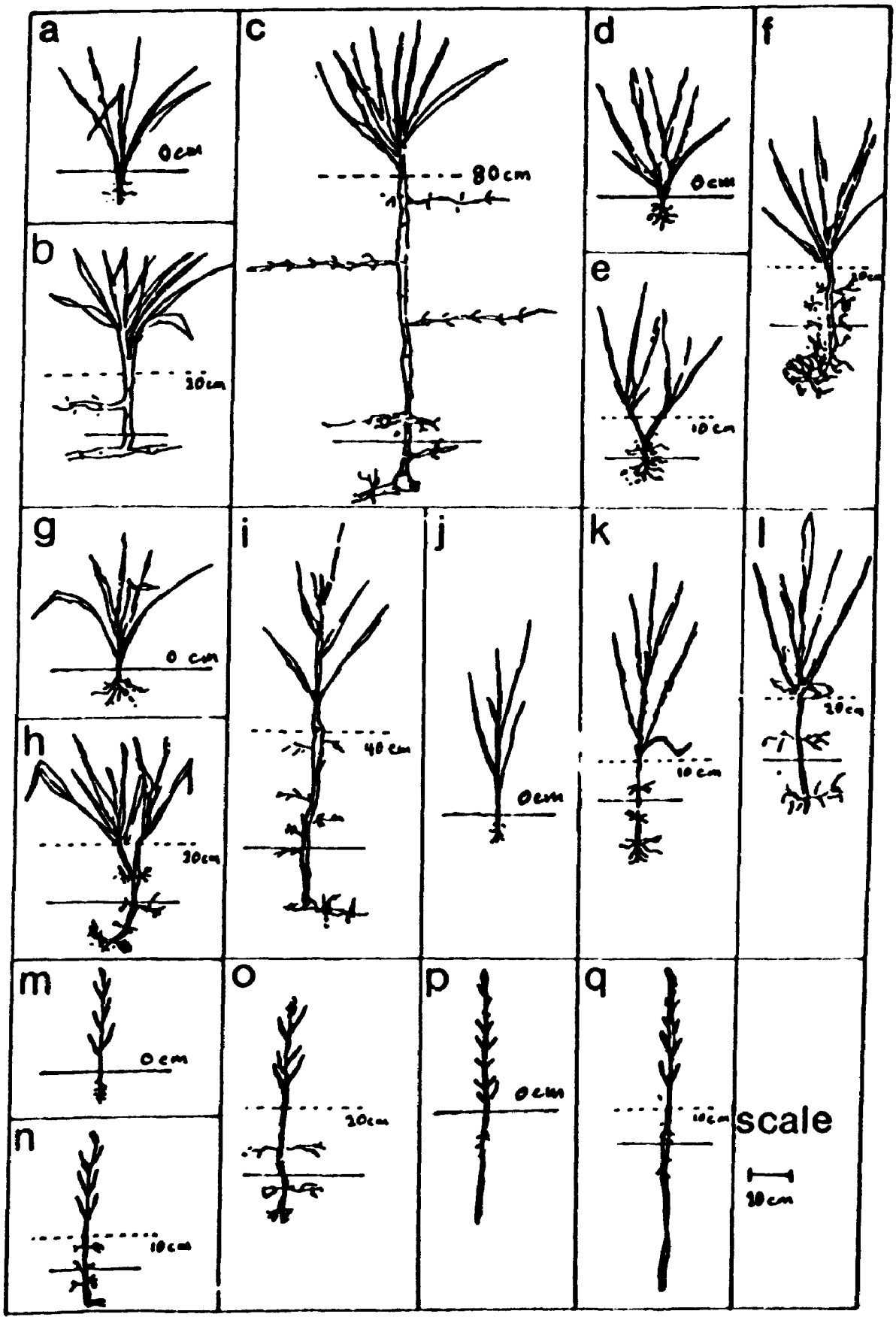
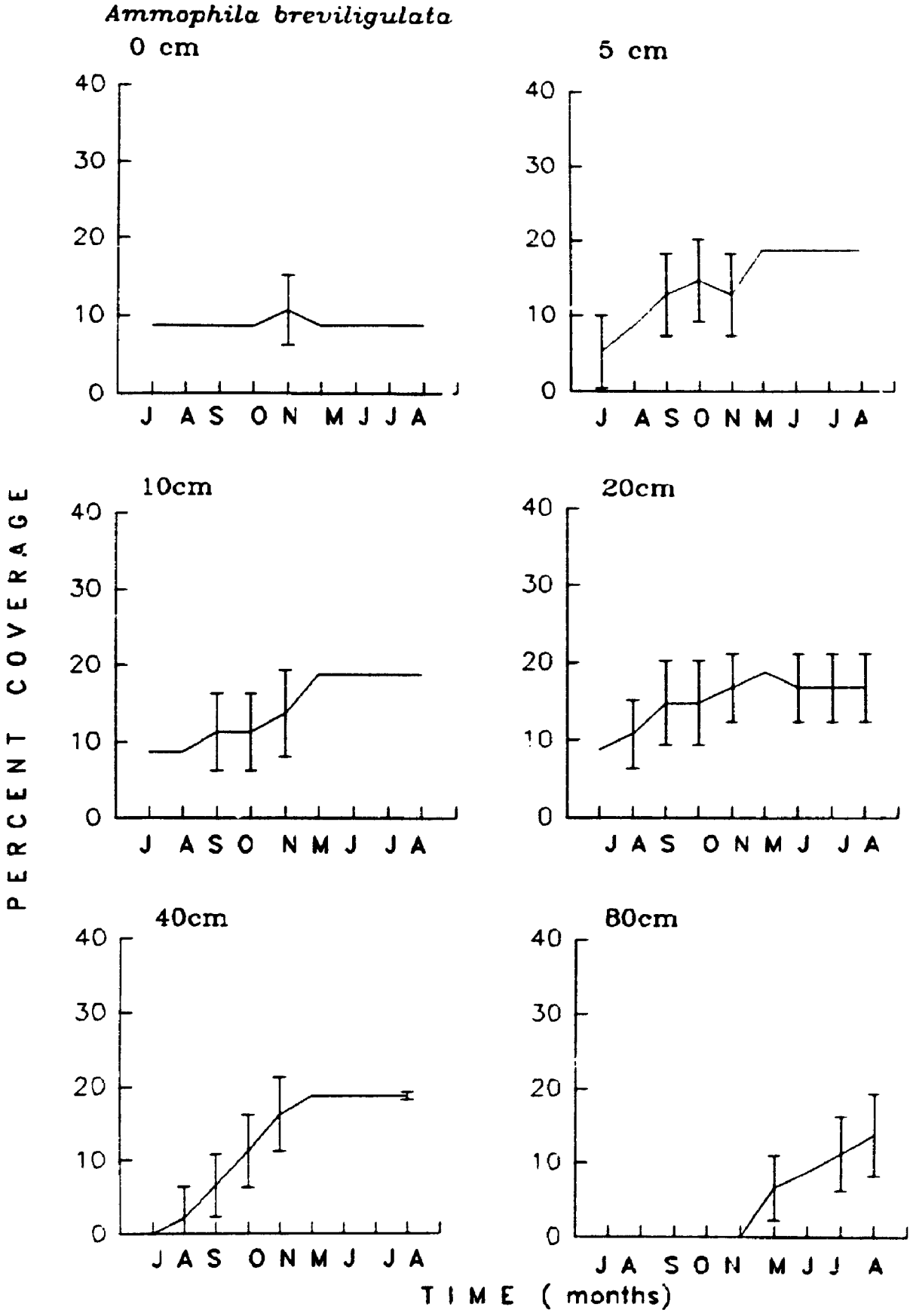

a
0cm
c
80cm
d
0cm
f
b
30cm
e
10cm
g
0 cm
i
40cm
j
0cm
k
10cm
l
20cm
h
20cm
m
0cm
o
20cm
p
0cm
q
10cm
scale
10cm
n
10cm

Figure 2.7 Mean (± SE) percent cover of *Ammophila breviligulata* as affected by different burial depths at Pinery Provincial park. The cover was recorded at monthly intervals from July to November 1992 and May to August 1993 N=24


Ammophila breviligulata
0 cm
5 cm
10cm
20cm
40cm
80cm
PERCENT COVERAGE
0
10
20
30
40
J A S O N M J J A
TIME (months)

Andropogon scoparius plants died in the 40 and 80 cm burial treatments. At 5, 10, and 20 cm burial depths the plants did not show any significant change in their percent cover as compared to control In buried plots, the dead leaves at the base of the plants were covered with sand and live shoots were more luxuriant than the control The cover value however, was similar to that of the control (Fig 2 8). *Calamovilfa longifolia* did not exhibit any enhanced growth in the 5 cm burial treatment but the 10 cm treatment showed a marked increase in the percent cover. The percent cover increased from about 16% in the control to about 45% in the 10 cm burial plot In the 20 cm burial treatment there was no increase in cover until June, 1993 and even then, it was similar to the control. In the 40 cm burial treatment, plants started to emerge in September, 1992 and continued to increase in cover to about 16% by August, 1993 *Calamovilfa longifolia* plants failed to emerge from 80 cm burial depths (Fig 2 9)

Buried plants of *Elymus canadensis* exhibited a slight increase in percent cover in the 5 and 10 cm burial plots as compared to the control. The percent cover for the control was about 10% while the buried plants had a cover of about 15% (Fig. 2.10). There was no emergence of *Elymus canadensis* in any of the higher burial depths (20, 40 and 80 cm)

Lithospermum carolinense did not show any marked differences between the control and the 5 cm burial treatment but there was a marked decrease in the percent cover of the 10 cm burial treatment (Fig. 2.11). The species did not emerge from the sand deposits when buried to depths of 20, 40, and 80 cm.

The control and 5 cm plants of *Poa compressa* did not exhibit any significant difference in the percent cover which was at around 15%, but there was a decline in the cover in the 10 and 20 cm plots (Fig. 2 12) There was no emergence of *Poa compressa* in any of the burial treatments above 20 cm

The scatter diagram of the six species in Experiment I showed four main groups on the bases of tolerance to burial in sand ,(i) *Calamovilfa longifolia*, (ii) *Ammophila breviligulata*, (iii) *Andropogon scoparius* and (iv) *Elymus canadensis*, *Lithospermum carolinense* and *Poa compressa* (Fig 2 13).

Ammophila breviligulata tolerated more burial depths than *Calamovilfa*

Figure 2.8 Mean (± SE) percent cover of *Andropogon scoparius* as affected by different burial depths at Pinery Provincial park The cover was recorded at monthly intervals from July to November 1992 and May to August 1993 N=24

Andropogon scoparius

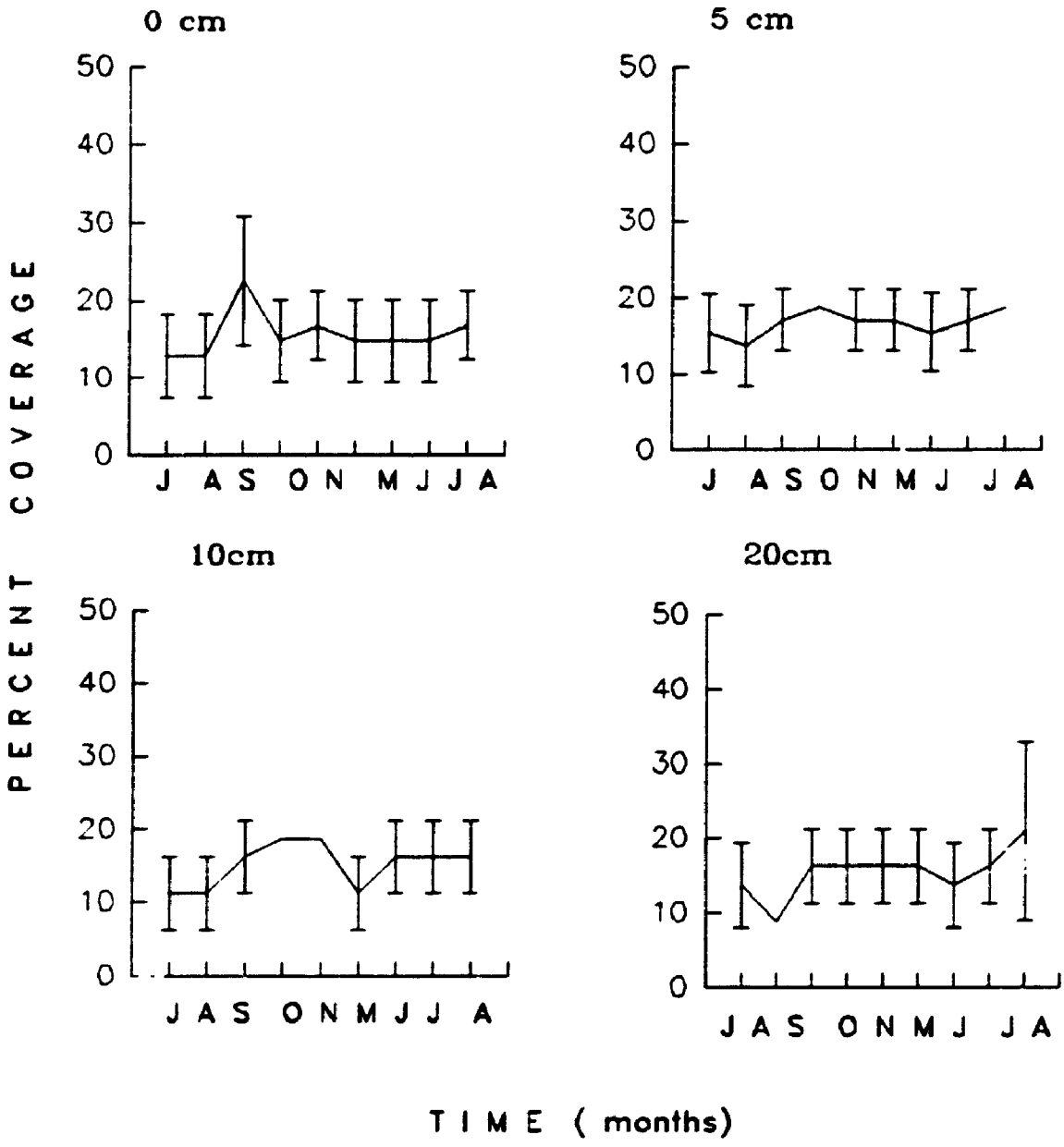


Figure 2.9 Mean (± SE) percent cover of *Calamovilfa longifolia* as affected by different burial depths at Pinery Provincial Park The cover was recorded at monthly intervals from July to November 1992 and May to August 1993 N=24

Calamovilfa longifolia

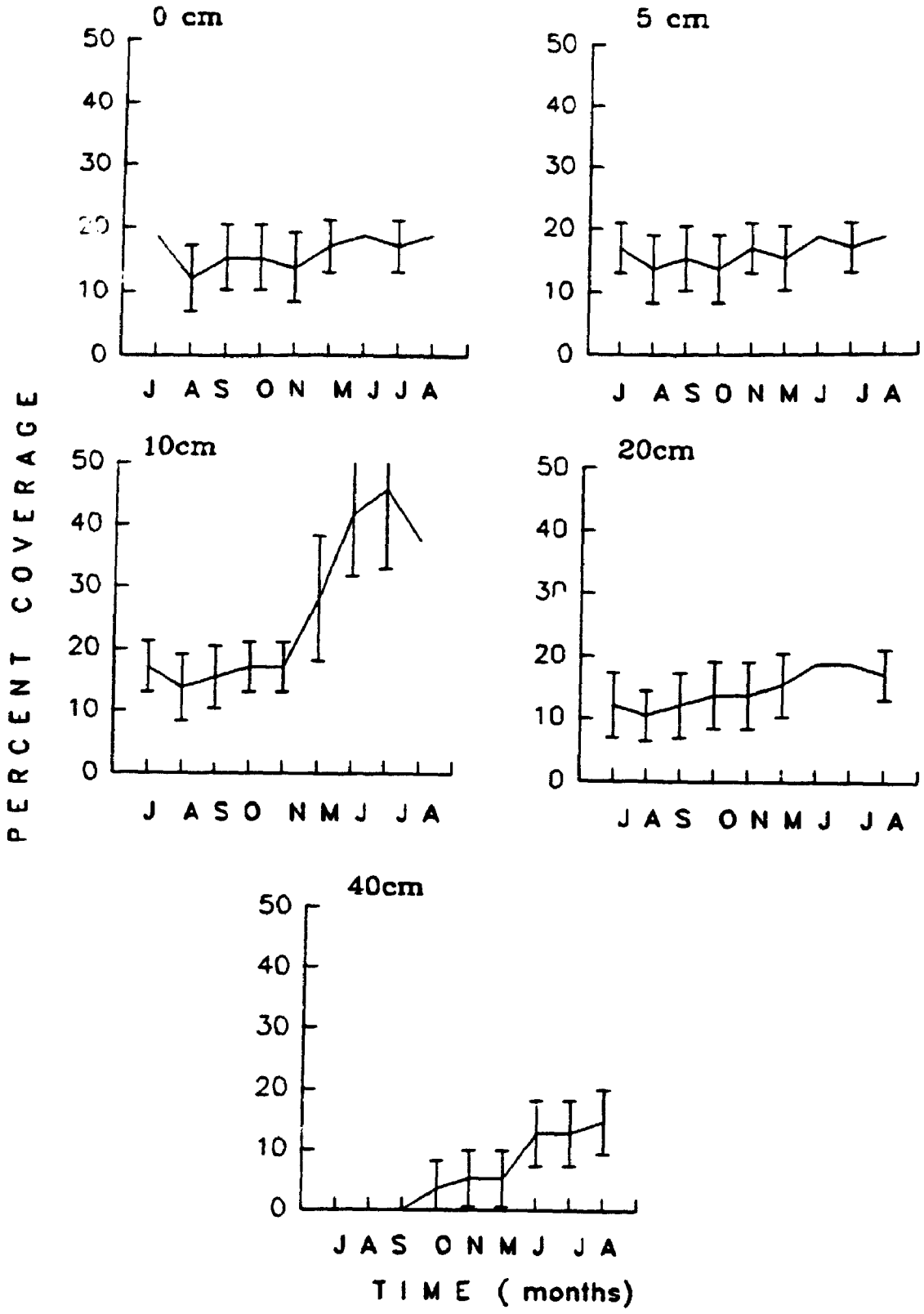


Figure 2.10 Mean (± SE) percent cover of *Elymus canadensis* as affected by different burial depths at Pinery Provincial park. The cover was recorded at monthly intervals from July to November 1992 and May to August 1993. N=24

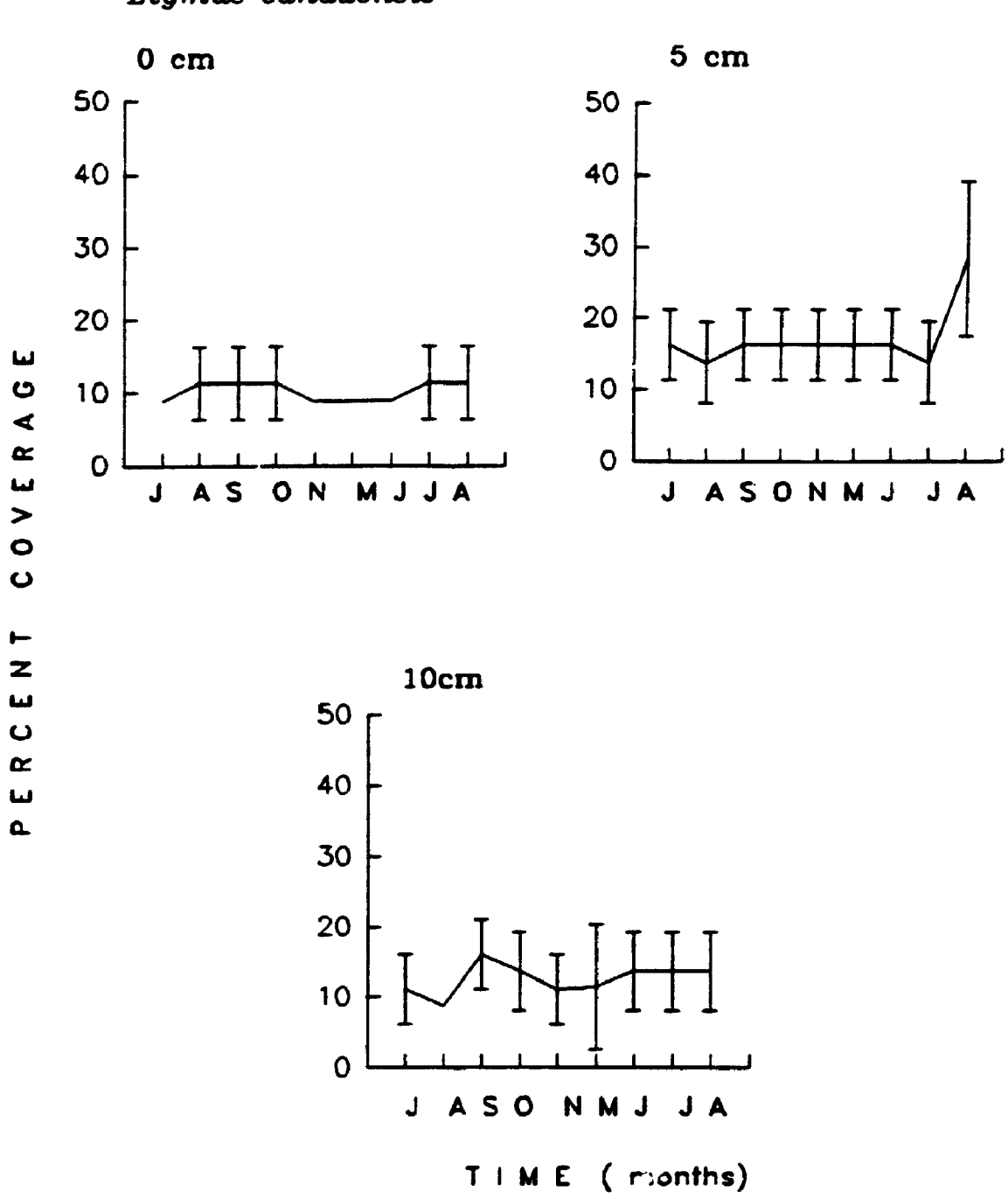

Elymus canadensis
0 cm
5 cm
10cm
50
40
30
20
10
0
J A S O N M J J A
PERCENT COVERAGE
TIME (months)

Figure 2.11 Mean (± SE) percent cover of *Lithospermum carolinense* as affected by different burial depths at Pinery Provincial park. The cover was recorded at monthly intervals from July to November 1992 and May to August 1993 N=24

Lithospermum caroliniense

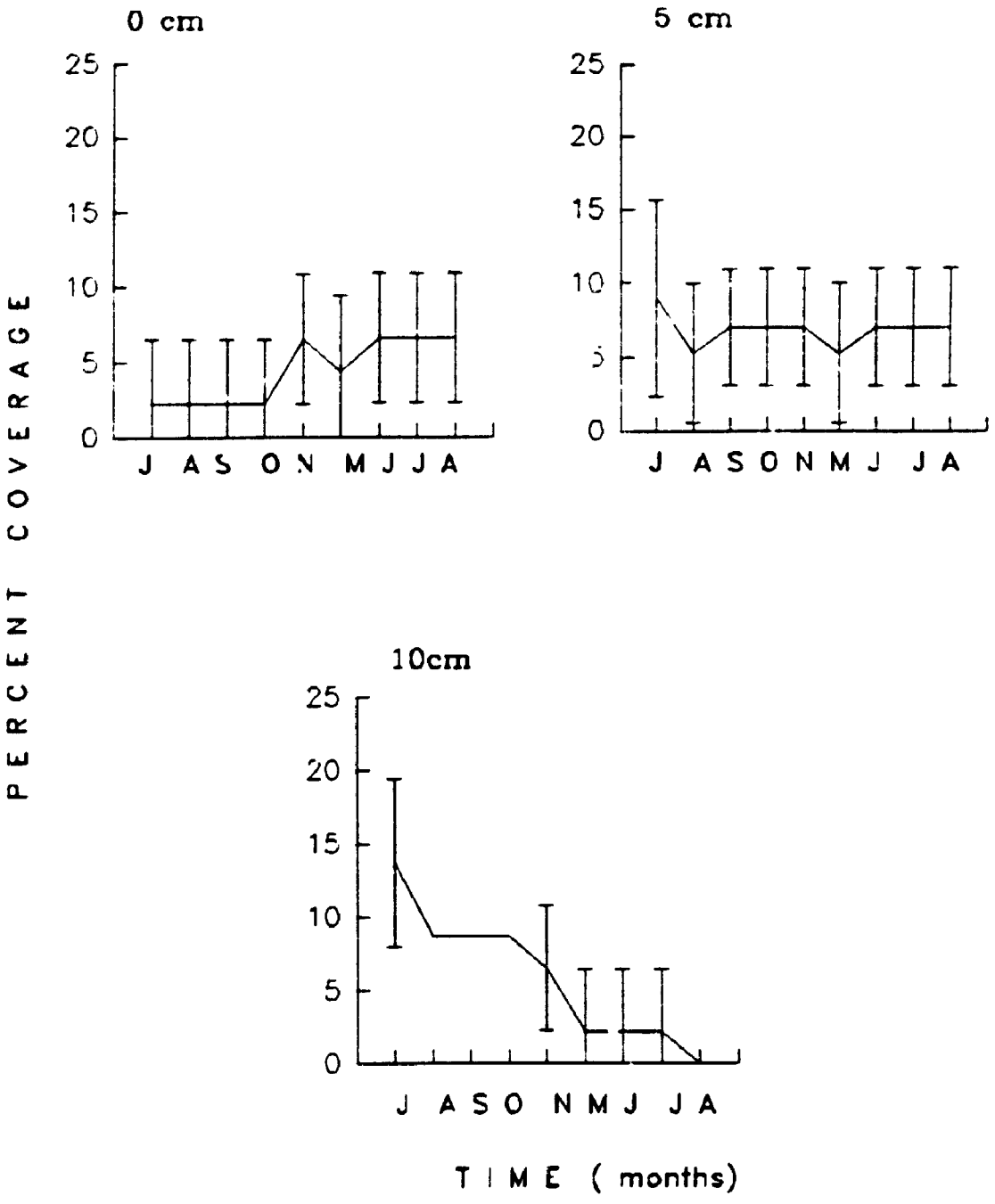


Figure 2.12 Mean (± SE) percent cover of *Poa compressa* as affected by different burial depths at Pinery Provincial Park. The cover was recorded at monthly intervals from July to November 1992 and May to August 1993. N=24

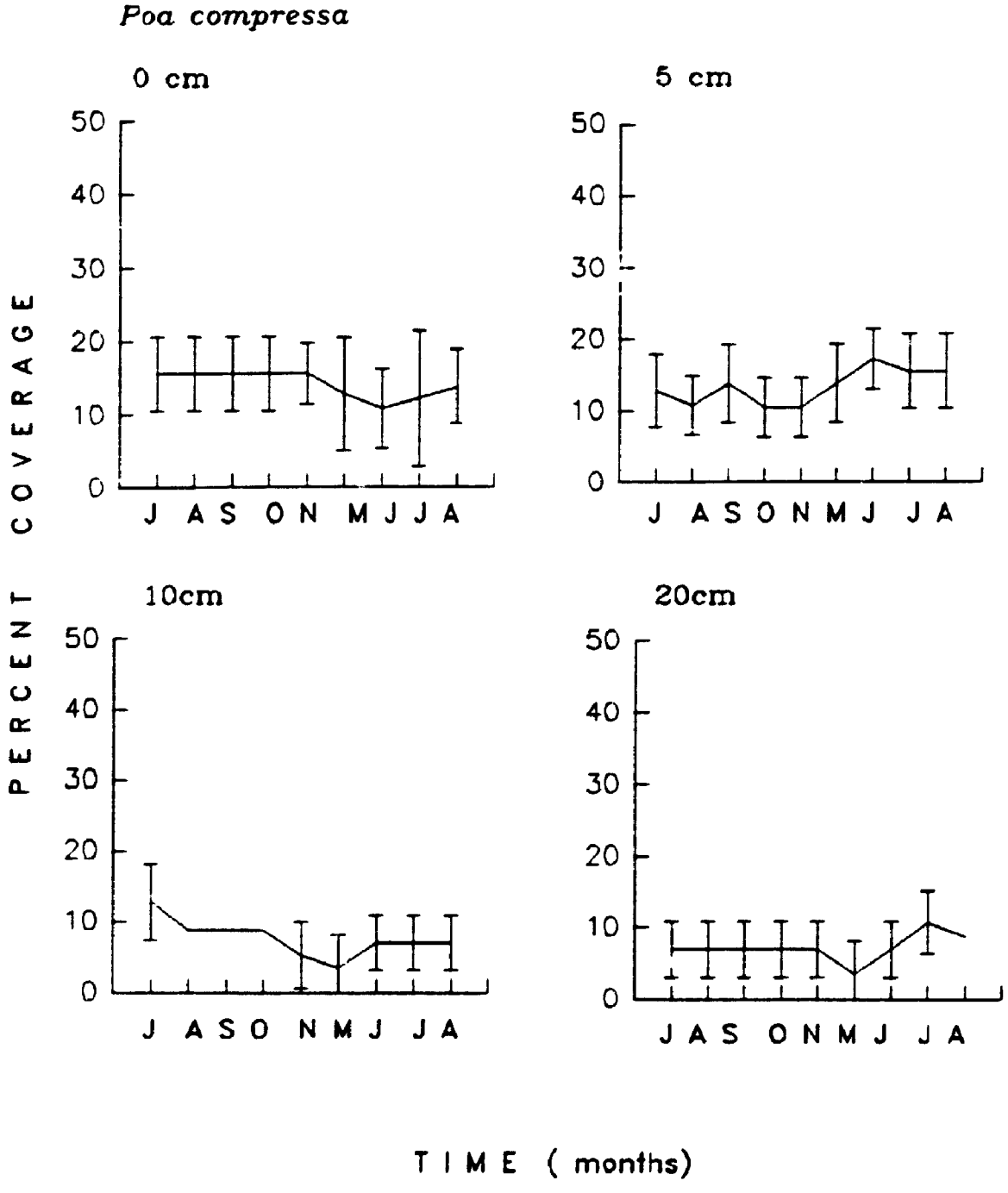
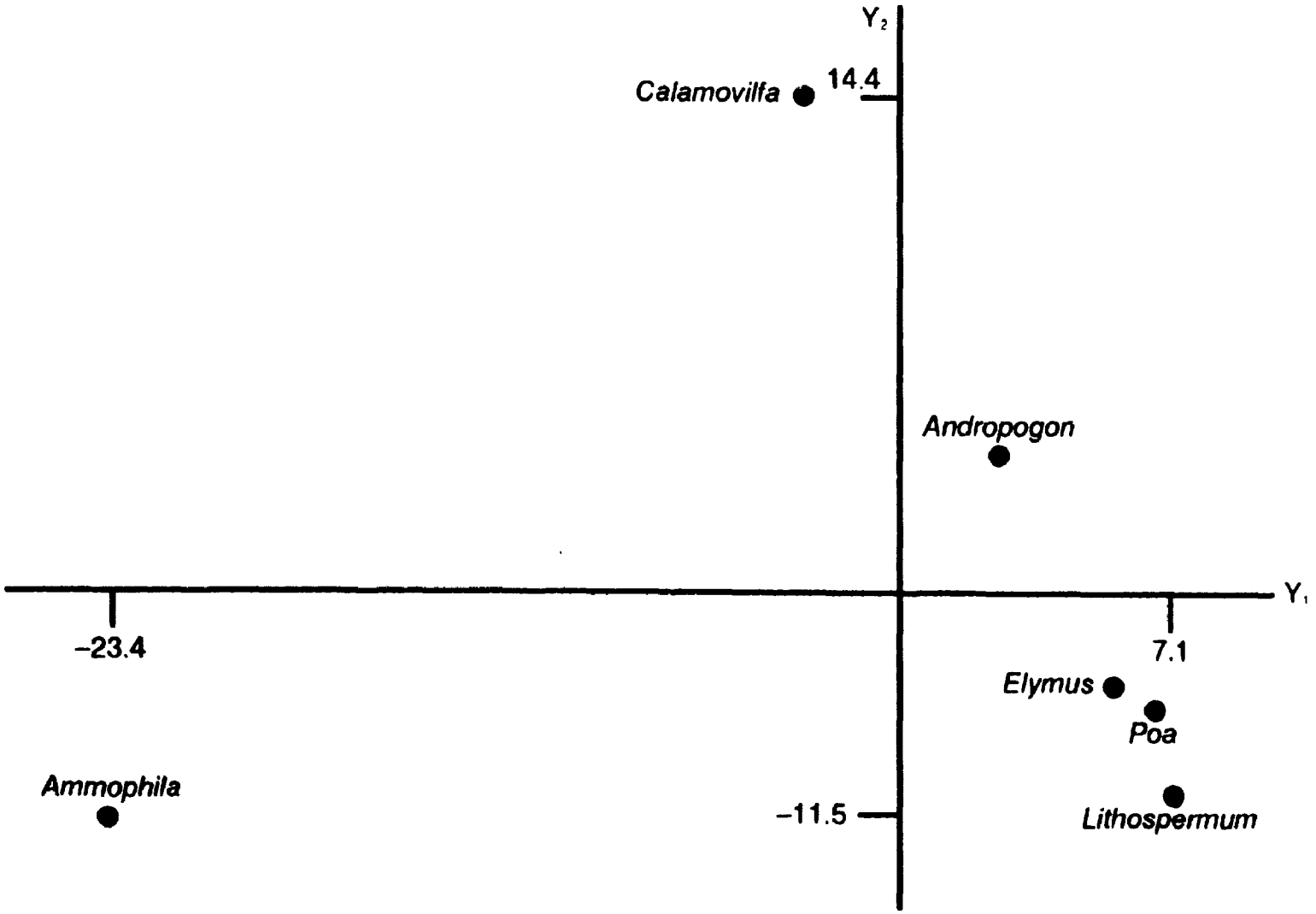

Poa compressa
0 cm
5 cm
10cm
20cm
PERCENT COVERAGE
50
40
30
20
10
0
J A S O N M J J A
J A S O N M J J A
J A S O N M J J A
J A S O N M J J A
TIME (months)

Figure 2.13 Scatter diagram showing the canonical group analysis of species distribution in relation to burial depth (cm) in an artificial burial experiment at Pinery Provincial Park. The first two axes of the ordination are plotted.


Y2
14.4
Calamovilfa
Andropogon
Y1
-23.4
7.1
Elymus
Poa
Ammophila
-11.5
Lithospermum

longifolia which in turn survived better than *Andropogon scoparius* The other three species were similar in their response to burial There was an increase in the total biomass of buried plants as compared to the control, except in the case of *Lithospermum carolinense* where there was no significant difference *Ammophila breviligulata* was the only species that emerged from the maximum burial depth of 80 cm In the 40 cm burial treatment *Ammophila breviligulata* and *Calamovilfa longifolia* were the only species that survived the episode *Lithospermum carolinense* and *Elymus canadensis* did not survive the 20 cm burial The control had the largest number of species and individuals, but the plants were much smaller than those in the buried treatments (Table 2 4)

The root/shoot ratio showed a general increase in the ratio with increased burial except in *Lithospermum carolinense* where there was a decrease in the root/shoot ratio Data for *Calamovilfa longifolia* were log transformed because it did not meet the assumptions of ANOVA in having equal variances according to Bartlett's test (Table 2 5)

The plants generally showed an increase in plant height when they were buried, especially in *Ammophila breviligulata* and *Elymus canadensis* in which the increase was linear and occurred in all treatments The data for *Calamovilfa longifolia* were also log transformed as in the case of the root/shoot ratio (Table 2 6)

2.3.3 Experiment II: Effects of artificial sand burial on species abundance

The analytical procedure (CONAPACK) used in this experiment was the same as that of Experiment I (see section 2.3 2 for details) The calculated Chi Square value was 28 7 and the Critical Table value was 66 3 The null hypothesis (H_o) was therefore accepted that the species at the experimental site were randomly distributed (Table 2 7)

In a similar way, the final readings were analyzed using CONAPACK to determine whether there was an association between various depths of sand burial in sand and species abundance The calculated Chi Square value was 137 3 and the

Table 2.4: Mean total plant dry weight (g) at final harvest of six common dune species, buried for two growing seasons to various depths (0, 5, 10, 20, 40, 80 cm) in sand at Pinery Provincial Park.

	Burial depths (cm)					
Species	0 (control)	5	10	20	40	80
Ammophila breviligulata	23.61a*	———	37.98ab	64.81bc	59.2c	82.0d
Calamovilfa longifolia	26.85a	44.58ab	86.74c	114.57d	65.96bc	-------
Andropogon scoparius	11.57a	35.25b	63.20c	77.20c	-------	-------
Elymus canadensis	5.55a	16.08ab	19.55b	20.86b	-------	-------
Poa compressa	2.29a	7.78b	16.20c	16.20c	-------	-------
Lithospermum caroliniense	2.57a	2.01a	1.73a	-------	-------	-------

* Values in the same row followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's range test.

——— Species was absent from plots in this treatment even before burial.

--------- Plants did not emerge, but were present before burial.

Table 2.5: Mean root-shoot ratio at final harvest of six common dune species, buried for two growing seasons to various depths (0, 5, 10, 20, 40, 80 cm) in sand at Pinery Provincial Park.

	Burial depths (cm)					
Species	0 (control)	5	10	20	40	80
Ammophila breviligulata	0.68a*	———	0.77a	1.51b	2.08c	2.62d
Calamovilfa longifolia‡	1.41a	1.65b	1.93cd	2.05d	1.81c	-------
Andropogon scoparius	0.63a	0.70a	0.64a	0.86b	-------	-------
Elymus canadensis	0.56ab	0.66b	0.40a	0.69b	-------	-------
Poa compressa	0.70a	0.66a	0.78a	1.24b	-------	-------
Lithospermum caroliniense	0.81	0.77a	0.733a	-------	-------	-------

* Values in the same row followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's range test.

——— Species was absent from plots in this treatment even before burial.

--------- Plants did not emerge, but were present before burial.

‡ Data were log transformed because of unequal variances.

Table 2.6: Plant height (in cm) at final harvest of six common dune species, buried to various depths (0, 5, 10, 20, 40, 80 cm) in sand at Pinery Provinvial Park.

	Burial depths (cm)					
Species	0 (control)	5	10	20	40	80
Ammophila breviligulata	63.62a*	———	72.25ab	86.82b	105.90c	146.70d
Calamovilfa longifolia‡	1.72a	1.72a	1.97b	2.05b	1.99b	-------
Andropogon scoparius	56.63a	79.62b	84.32b	79.03b	-------	-------
Elymus canadensis	43.75a	64.65b	67.55bc	84.40c	-------	-------
Poa compressa	2.29a	7.78b	16.20c	7.94b	-------	-------
Lithospermum caroliniense	39.90a	38.63a	37.93a	-------	-------	-------

* Values in the same row followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's range test.

——— Species were absent from plots in this treatment even before burial.

------- Plants did not emerge, but were present before burial.

‡ Data were log transformed, because variances were unequal.

Table 2.7: Canonical correlations and cumulative percentages of data explained by each partition.

Treatment	Partition	Canonical correlations	Cumulative percentages
Pre-burial	1	0.28	55.14
	2	0.20	81.81
	3	0.15	96.41
	4	0.07	100.00
	$\chi^2 = 28.73 < \chi^2\ 0.05, 36\ (66.34)$ Null hypothesis that species distribution and abundance are independent is accepted. Therefore, the species are randomly distributed.		
Post-burial	1	0.71	43.18
	2	0.59	74.33
	3	0.50	95.90
	4	0.22	100.00
	$\chi^2 = 137.34 > \chi^2\ 0.05, 36\ (66.34)$ Null hypothesis that burial depth and species distribution are independent is rejected. Therefore, species distribution is dependent on burial depth.		

Critical Table value was 66 3 Thus, the null hypothesis was rejected that there was an association between species abundance and burial depth (Table 2 7).

Agropyron psammophilum had about 28% cover in the plots before burial. Following burial, plant cover declined initially in the first 2 or 3 months followed by an increase For example, in the 5 cm burial treatment the cover went down to about 10% but by August, 1992, 3 months after burial, the cover had increased to 33%. In the 10 cm burial plot the plants attained the original cover by September, 1992, 4 months after burial In the 15 and 20 cm burial treatments the initial cover dropped down to about 5%, but by September, 1992 the plants had increased in cover to about 33% (Fig 2 14)

Panicum virgatum exhibited a stimulation in growth due to burial treatments. The cover value before burial was about 28% in the autumn of 1992. The plants started to emerge from control and burial treatments in May, 1993 The coverage was low initially in all plots including the control but it continued to increase until the burial plots of 5, 10, 15 and 20 cm had not only regained but had surpassed the control by September, 1993 (Fig 2 15)

Cakile edentula did not show a marked difference as a result of burial. In the 5 cm burial treatment there was an increase in the percent cover about a month after burial, but it was not different In the 10 cm burial there was an initial decrease in the percent cover, but the plants regained their original cover of about 8% by June, 1992. The decrease in cover in August and September was primarily due to natural senescence of the plants There was no emergence of *Cakile edentula* plants in 15 and 20 cm burial treatments (Fig 2.16)

The preburial cover of *Corispermum hyssopifolium* was about 18%. The burial treatment of 5 cm decreased the cover to about 5% initially but the plants recovered by August, 1992 and had increased their cover to about 15% (Fig. 2.17). *Corispermum hyssopifolium* did not emerge in any of the higher depths of burial.

Euphorbia polygonifolia responded to burial in a manner similar to that of *Corispermum hyssopifolium* The 5 cm burial completely overwhelmed the plants and cover was reduced to almost 0%. However, the plants started to emerge soon after the treatment and by August, 1992 attained a cover of about 8% (Fig. 2. 18). The plants

Figure 2.14 Mean (± SE) percent cover of *Agropyron psammophilum* as affected by different burial depths at Port Burwell Provincial Park The cover was recorded at monthly intervals from May to September 1992 N=40

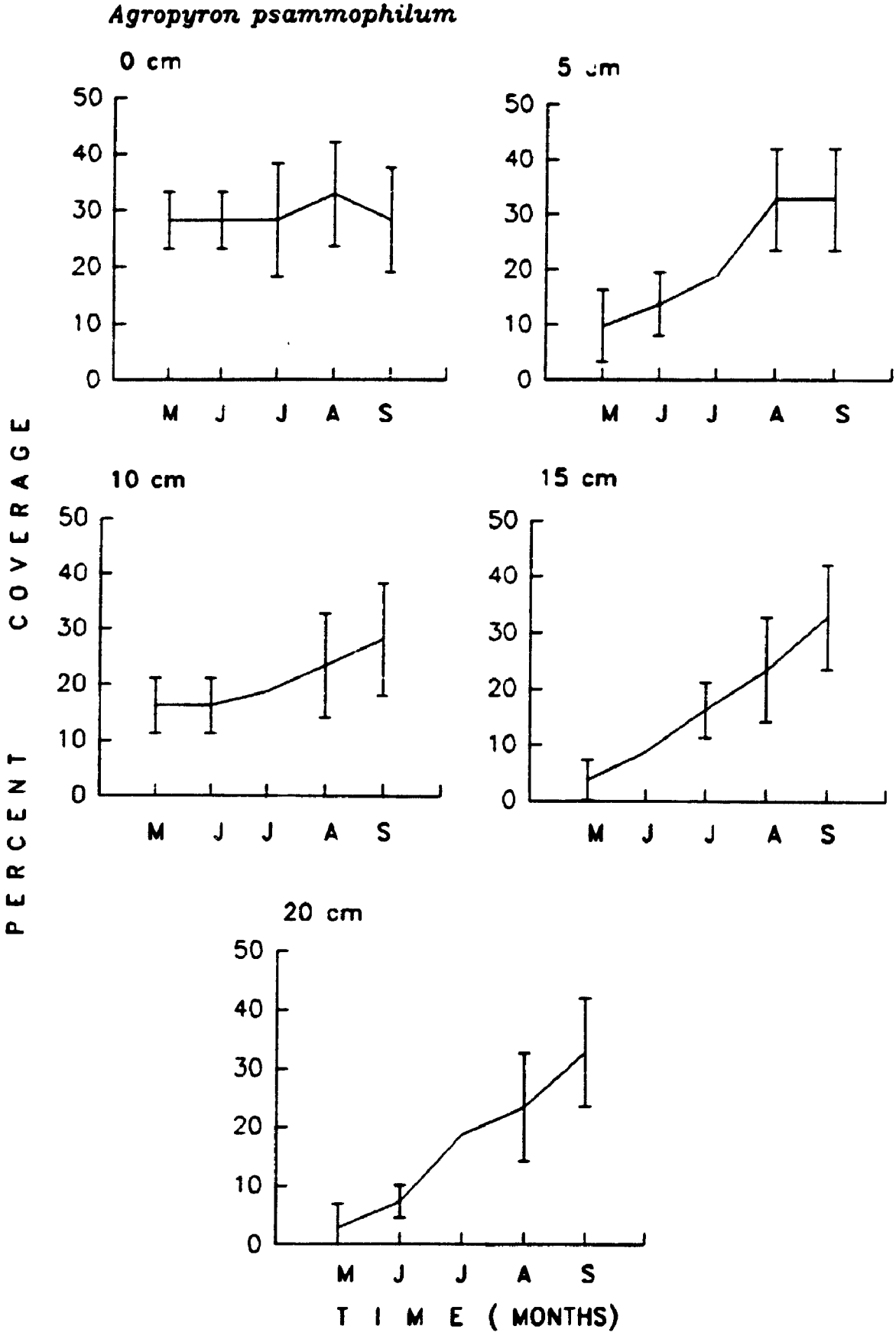

Agropyron psammophilum
0 cm
5 cm
10 cm
15 cm
20 cm
PERCENT COVERAGE
50
40
30
20
10
0
M J J A S
TIME (MONTHS)

Figure 2.15 Mean (± SE) percent cover of *Panicum virgatum* as affected by different burial depths at Port Burwell Provincial Park. The cover was recorded at monthly intervals from May to September 1992. N=40

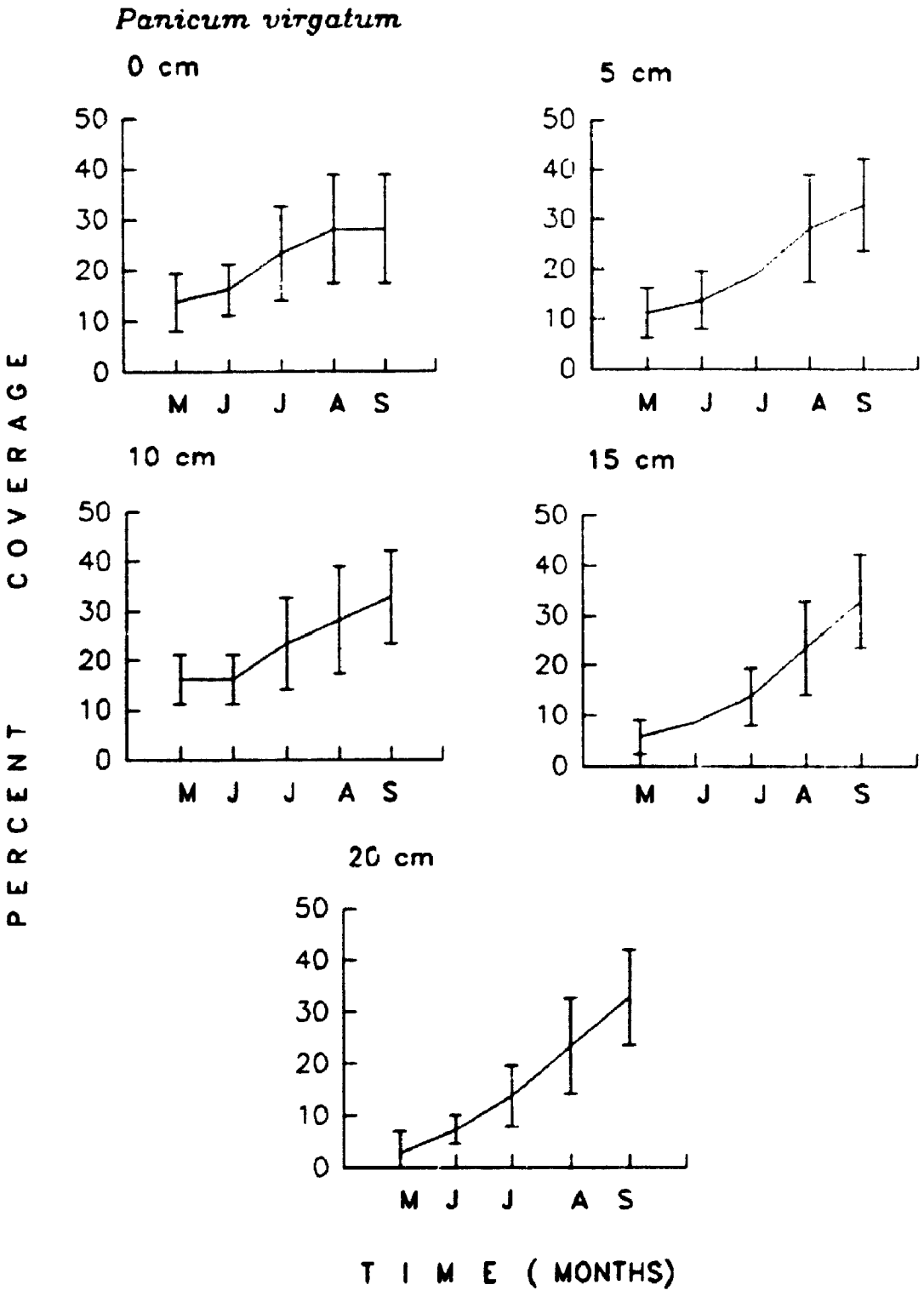

Panicum virgatum
0 cm
5 cm
10 cm
15 cm
20 cm
PERCENT COVERAGE
50
40
30
20
10
0
M J J A S
TIME (MONTHS)

Figure 2.16 Mean (± SE) percent cover of *Cakile edentula* as affected by different burial depths at Port Burwell Provincial Park. The cover was recorded at monthly intervals from May to September 1992. N=40

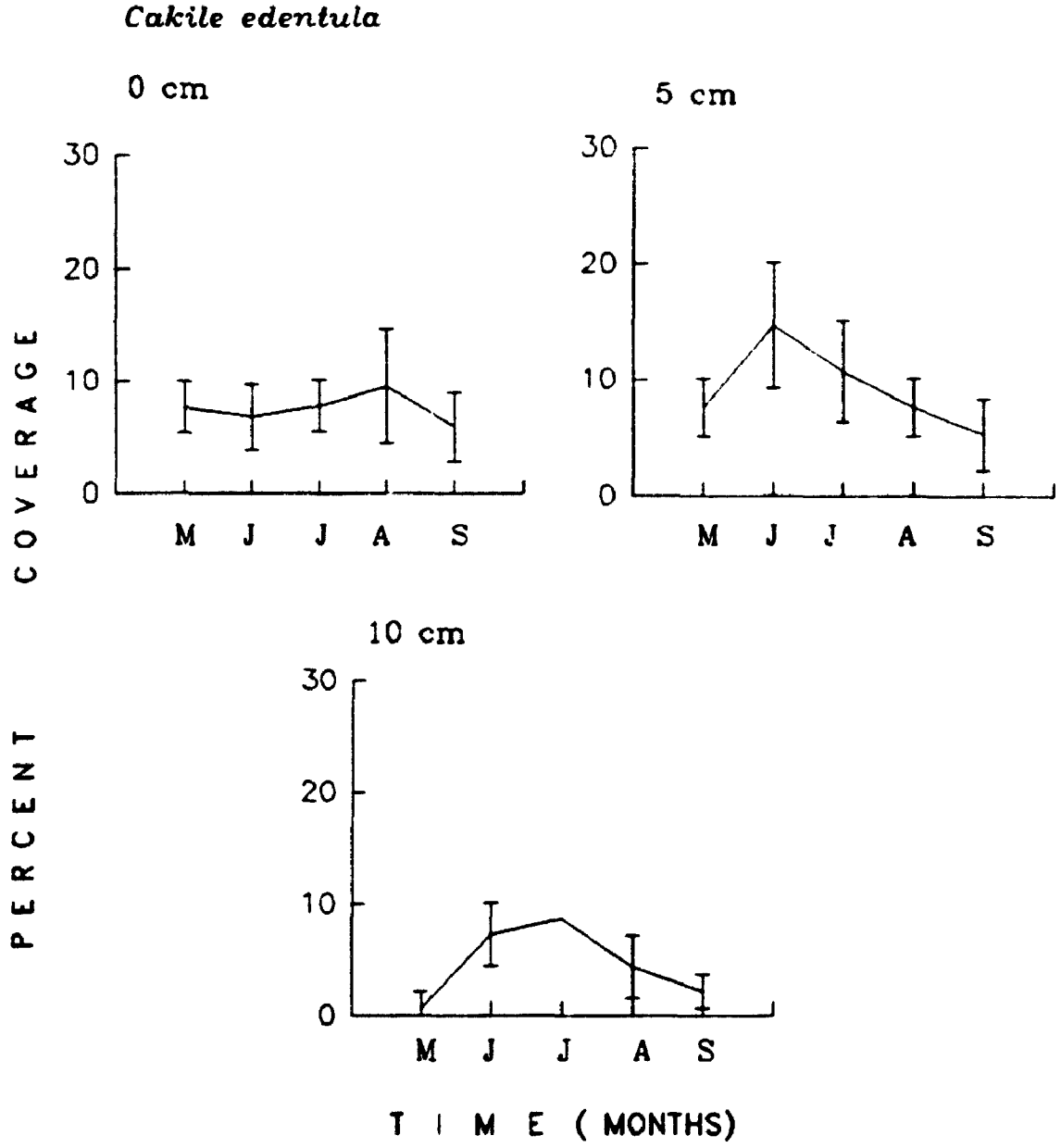

Cakile edentula
0 cm
5 cm
10 cm
30
20
10
0
M J J A S
PERCENT COVERAGE
TIME (MONTHS)

Figure 2.17 Mean (± SE) percent cover of *Corispermum hyssopifolium* as affected by different burial depths at Port Burwell Provincial Park. The coverage was recorded at monthly intervals from May to September 1992 N=40

Corispermum hyssopifolium

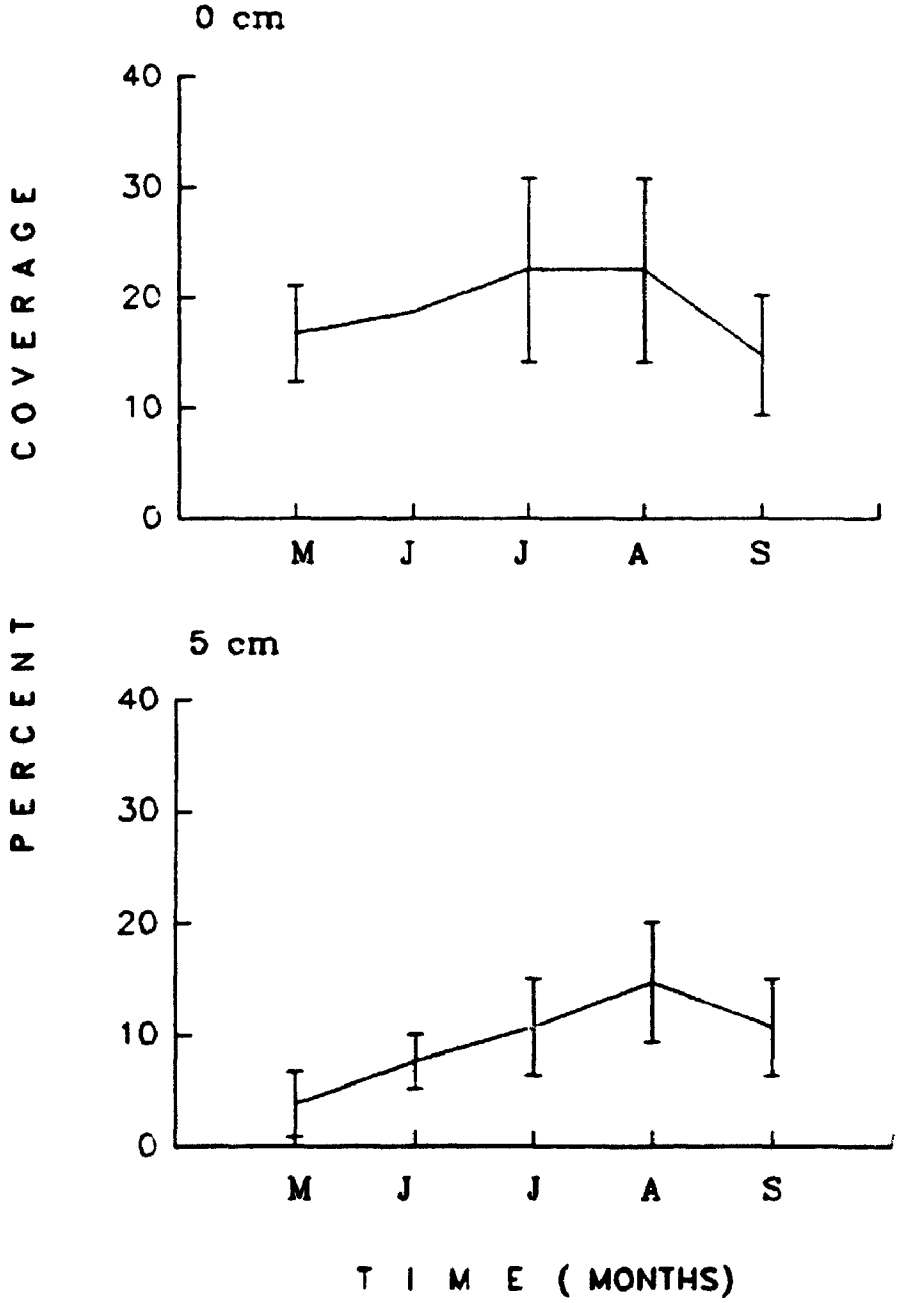
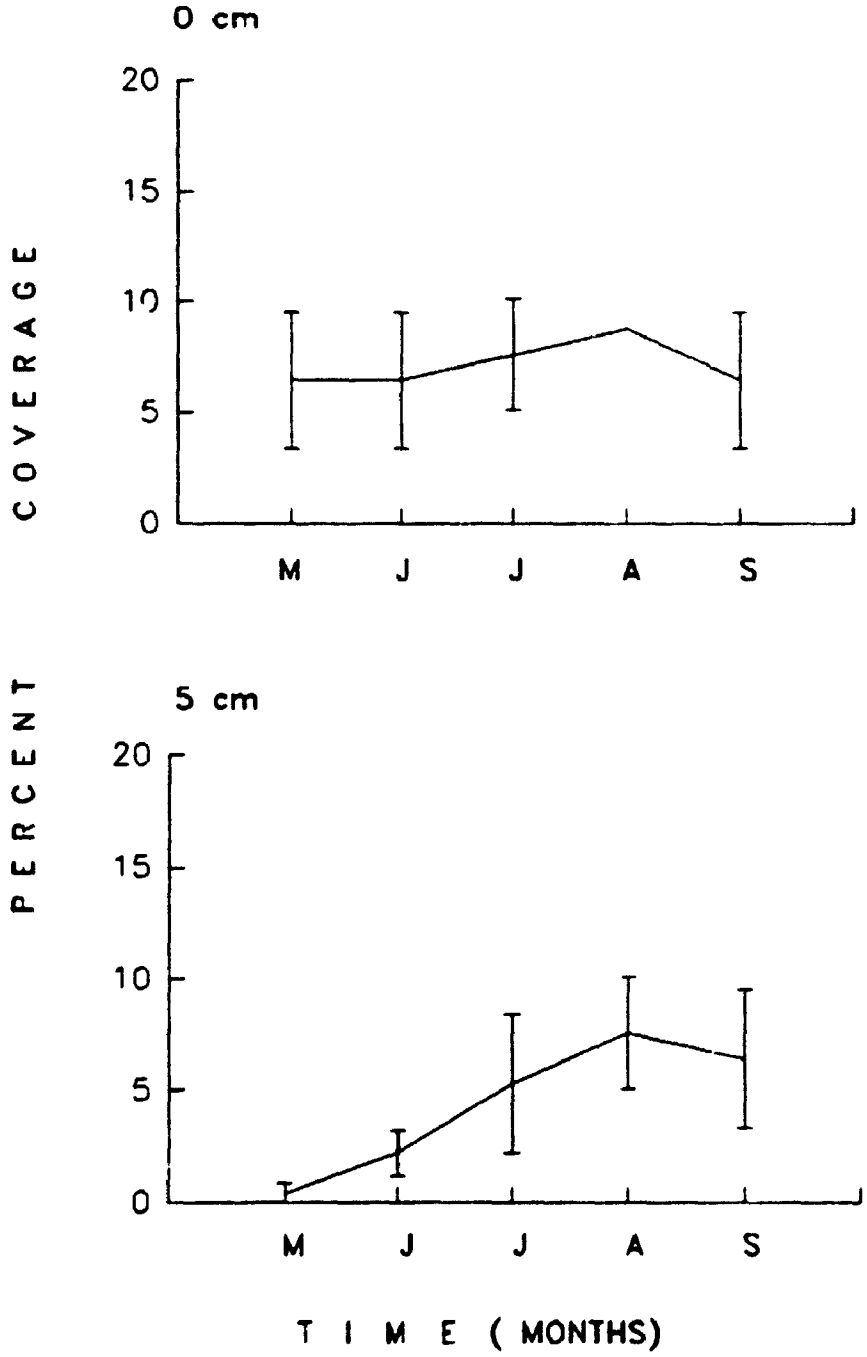


Figure 2.18 Mean (± SE) percent cover of *Euphorbia polygonifolia* as affected by different burial depths at Port Burwell Provincial Park. The cover was recorded at monthly intervals from May to September 1992. N=40

Euphorbia polygonifolia



failed to emerge from burial depths of 10, 15 and 20 cm

Melilotus alba responded remarkably well to the burial treatments The plants had a cover value of about 10% prior to burial The buried plants showed an increase in percent cover For example, in the 5, 10 and 15 cm burial treatments there was an initial decline in the cover values but by August, 1992, about 3 months after burial the plants had recovered and had a 20% cover (Fig 2 19). The plant did not emerge in the 20 cm burial plots

Strophostyles helvola also showed improved growth after the burial treatment as compared to control which had a cover of about 10% After an initial decline in cover, the buried plants showed a big increase in cover by August, 1992 (Fig. 2.20). The plant did not survive the 15 and 20 cm burial treatments.

Xanthium strumarium showed a big response to burial treatments. Plants in the control treatment had a cover of only about 10% Burial treatments stimulated its growth For example, by September, the 5 cm burial treatment had 20% cover which increased to about 33% in the 10 cm burial plots Plants buried to 15 cm depths had a cover value of 28% which was only slightly lower than the 10 cm burial treatment (Fig 2 21) Plants in the 10 and 15 cm burial depths had an initial decline in their cover but they sh ed a quick recovery However the plants failed to emerge from the 20 cm burial treatment

The scatter diagram of the eight species in Experiment II showed three main groups on the bases of tolerances to burial depth in sand, (i) *Agropyron psammophilum* and *Panicum virgatum*, (ii) *Melilotus alba*, *Xanthium strumarium* and *Strophostyles helvola* and (iii) *Cakile edentula*, *Corispermum hyssopifolium* and *Euphorbia polygonifolia* (Fig 2 22) The two perennial grasses, *A. psammophilum* and *P. virgatum* tolerated greater amounts of burial in sand than the annuals and the one biennial (*Xanthium strumarium*)

Figure 2.19 Mean (± SE) percent cover of *Melilotus alba* as affected by different burial depths at Port Burwell Provincial Park. The cover was recorded at monthly intervals from May to September 1992. N=40

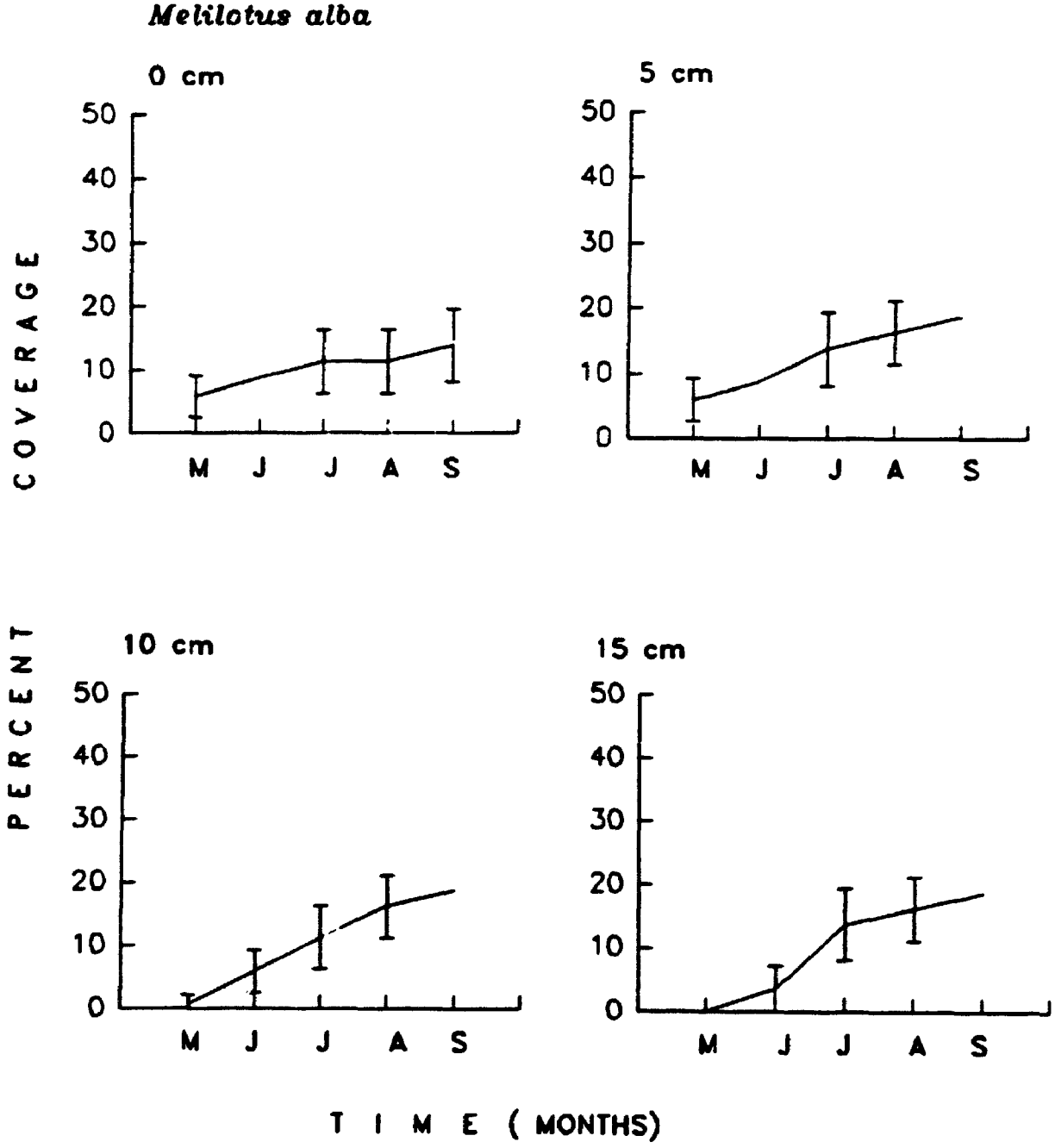

Melilotus alba
0 cm
5 cm
10 cm
15 cm
COVERAGE
PERCENT
50
40
30
20
10
0
M J J A S
TIME (MONTHS)

Figure 2.20 Mean (± SE) percent cover of *Strophostyles helvola* as affected by different burial depths at Port Burwell Provincial Park. The cov

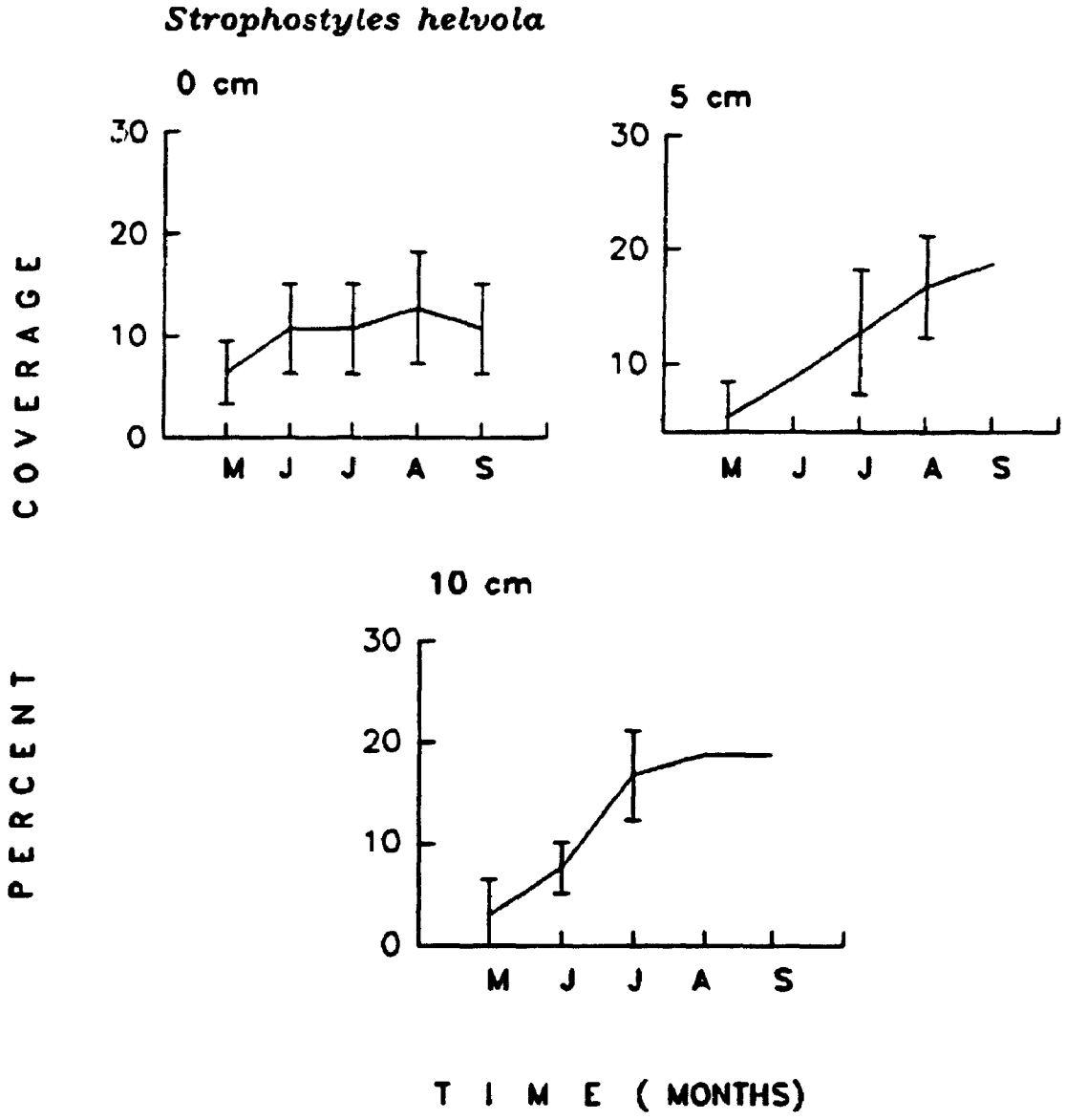

Strophostyles helvola
0 cm
5 cm
10 cm
30
20
10
0
M J J A S
PERCENT COVERAGE
TIME (MONTHS)

Figure 2.21 Mean (± SE) percent cover of *Xanthium strumarium* as affected by different burial depths at Port Burwell Provincial Park. The cover was recorded at monthly intervals from May to September 1992. N=40

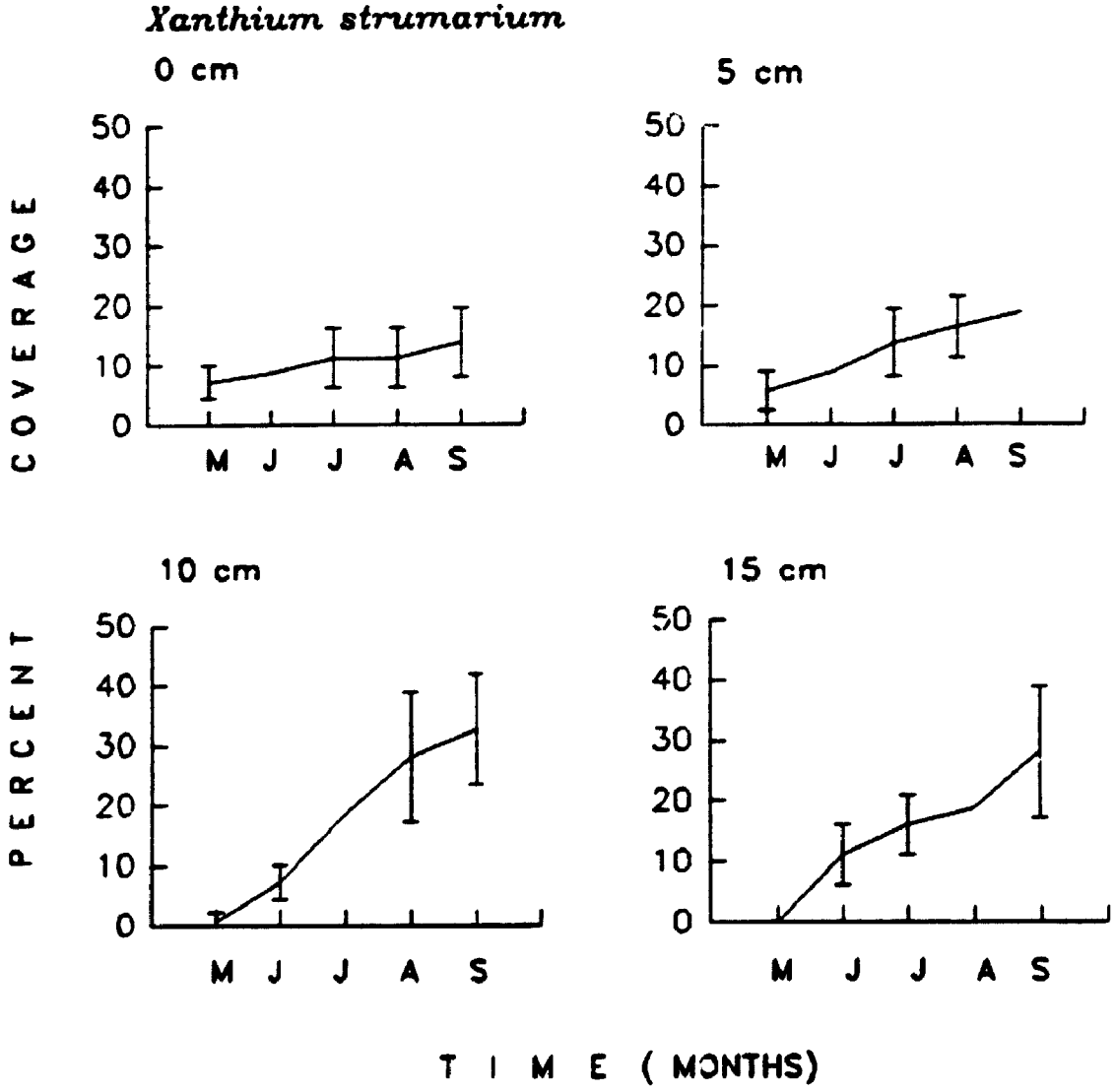
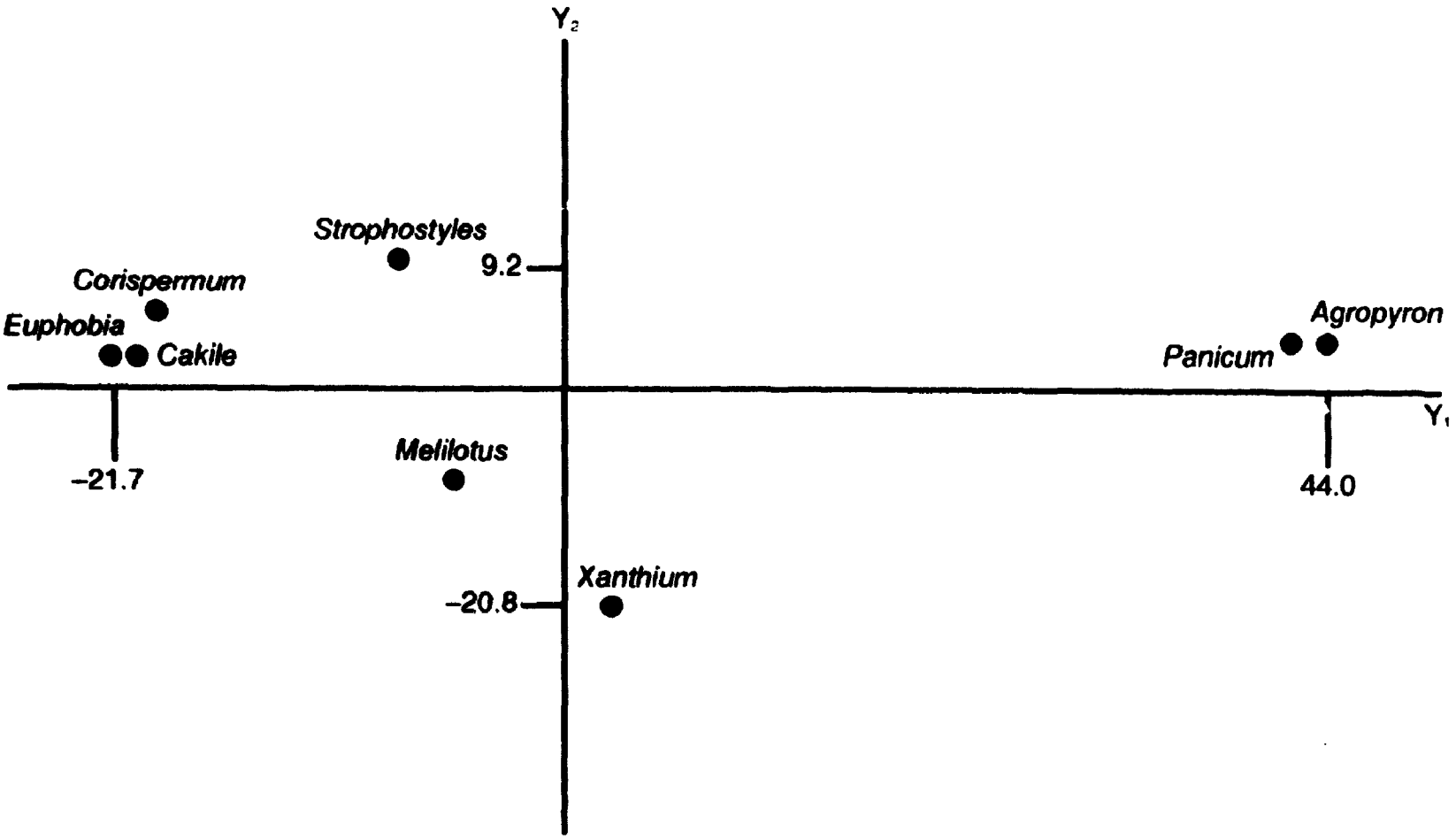

Xanthium strumarium
0 cm
5 cm
10 cm
15 cm
COVERAGE
PERCENT
50
40
30
20
10
0
M J J A S
TIME (MONTHS)

Figure 2.22 Scatter diagram showing the canonical group analysis of species distribution in relation to burial depth in an artificial burial experiment at the Port Burwell Provincial Park The first two axes of the ordination are plotted


Y2
Strophostyles
9.2
Corispermum
Euphobia
Cakile
Agropyron
Panicum
Y1
Melilotus
-21.7
44.0
Xanthium
-20.8

2.4 Discussion

The sand dune systems of the Great Lakes shorelines are dynamic Considerable movement of sand occurred during the years of 1991, 1992 and 1993 in different microhabitats The amount of sand movement at each stake, showed that there was erosion of sand at the crests except in areas with plant cover The amount of sand movement decreased at the upper and lower slopes (leeward) of the first dune ridge and in the slack Generally, sand accretion was higher in microsites with vegetation than those without, primarily due to erosion of sand which was prevalent in open areas without vegetation Open areas were created by excessive sand accretion that caused mortality of plants

The relevé ordination showed a clear correlation between sand movement, topography and the spatial distribution of species. For example, *Cakile edentula*, *Artemisia campestris* and *Corispermum hyssopifolium* were mainly located on the beach and open areas with low sand movement These species are short-lived annuals or biennials with opportunistic life history and high risk of seedling mortality. Stairs (1986) and Payne (1984) showed that 1-20% of the seedlings may survive to reproduce depending on the amount of sand movement and other environmental factors, such as moisture and temperature.

In regions of medium sand movement (accretion and erosion) *Andropogon scoparius* and *Juniperus communis* were abundant on the south slope of the first dune ridge These species are considerably taller than annuals and are both perennials. Sand accretion did not completely cover these plants so that they were able to survive the episode However, the two species did not survive in locations where they were completely covered

In regions of high sand movement *Ammophila breviligulata* and *Calamovilfa longifolia* were abundant Both species are important builders of the Great Lakes sand dunes and can withstand large amounts of sand burial (Maun and Lapierre, 1984, Eldred and Maun, 1982) Both of these species are rhizomatous perennial grasses with plenty of stored food which makes it possible for them to emerge from burial

conditions

This study shows that some species like *Ammophila breviligulata* thrive better than others under conditions of high sand accumulation However, under such conditions the cover of species like *Calamovilfa longifolia* and *Andropogon scoparius* was markedly reduced Barbour *et al.*, (1984) pointed out that it was not clear which species merely tolerate burial in contrast to others that required burial for maximum growth and completion of their life cycle

Controlled experiments revealed that the results were consistent with the study on relevé ordination The species can be classified into three categories according to their tolerance limits to sand accretion First category consisted of species such as *Ammophila breviligulata*, *Calamovilfa longifolia*, *Agropyron psammophilum* and *Panicum virgatum* which increased in relative abundance and were able to withstand 20 to 80 cm sand deposition *Ammophila breviligulata* was the only species that became abundant in plots receiving 80 cm sand deposition. In fact it can withstand up to 120 cm of sand accretion (Maun and Lapierre, 1984) It has been suggested that recurrent sand burial was essential for the maintenance of vigour in this species (Olson, 1958a) According to Eldred and Maun (1982), *Ammophila breviligulata* exhibited a decline in vigour in microhabitats with low sand accretion, which was manifested by a reduction in culm density, dry weight per shoot, and number of panicles m^{-2}. Maun (1993) further suggested that in areas where *Ammophila breviligulata* was declining, its minor associate, *Calamovilfa longifolia* became more abundant in the community. *Calamovilfa longifolia* could probably withstand up to 60 cm of burial, but not as much as 80 cm. *Calamovilfa longifolia* establishes exclusively from seeds as opposed to rhizome fragments and was a dominant dune builder in areas with less rapid or little sand deposition (40 to 60 cm per year) (Maun, 1993). *Agropyron psammophilum* and *Panicum virgatum* increased in abundance as the sand deposition per plot increased to 20 cm. Second category was that of annual and biennial species which occurred in large numbers in areas of low or medium sand deposition and their abundance decreased with an increase in sand deposition above their threshold for survival. The third category was of species such as *Lithospermum carolinense* which did not conform to the pattern shown by the other species,

probably because it could only withstand very small amounts of gradual sand accretion rather than the one time deposition imposed on plants in this experiment This could explain why this species was not found on accreting sites

In the initial stages of burial there was a negative impact on plant growth on all buried plants but depending on the burial depth, they started to recover especially if the burial depth, was not more than the threshold for survival Harris and Davy (1988) showed that the energy is diverted from buried leaves, stems and roots to the shoot apices of plants and the plant emerges and exposes its photosynthetic structures to sunlight, thus supporting growth There was a wide range of tolerance among various species to sand burial, but if they survived the burial depth, they invariably increased their total biomass, root/shoot ratio, and plant height This finding agrees with the work done by many other researchers (Sykes and Wilson, 1990, Harris and Davy, 1986; Maun and Lapierre, 1984, Maun and Riach, 1981, and Disraeli, 1984).

In conclusion, sand movement is an important factor in the distribution of plant species in sand dunes. My study has clearly shown that the ability of the species to withstand sand burial played a significant role in species distribution. The sand movement was variable in different microsites and depended on the distance from the lake, structure of dune, type of vegetation, the amount of sand deposition, and the time of the year. Further research is needed to determine the stage of growth at which plants are most vulnerable to sand accretion.

CHAPTER THREE

ECOPHYSIOLOGICAL RESPONSE TO SAND ACCRETION UNDER NATURAL CONDITIONS

3.1 Introduction

Plants growing on coastal and lacustrine sand dunes are exposed to a number of extreme conditions such as, high wind velocities, drastic temperature fluctuations, low water-holding capacity and organic matter content, high potential evapo-transpiration, burial in sand, salt spray, sand blast and shifting sandy substrate (Olson, 1958a,b, Baldwin and Maun, 1983, Mcleod and Murphy, 1983; Maun, 1985). Sand accretion can be quite high, especially on the foredunes and lee slopes of dune ridges (Maun and Lapierre, 1984) For example, upto 30 cm annual sand accretion may occur along both Lake Michigan (Olson, 1958) and the Atlantic Coast of North Carolina (van der Valk, 1974). sand accretion of 9 cm per year on the high beach along Lake Huron reported by Maun (1985) and that of 20 cm over two weeks along Lake Erie reported by Yanful (1988).

Sand movement (accretion and erosion) is a composite phenomenon. Apart from its direct mechanical effects of burial and erosion, it alters the amount of nutrients and moisture, probably modifies soil aeration in the surface layers and reduces or eliminates competition between plant species (Marshall, 1965). Sand accretion affects seedling establishment and adult populations in different ways (Barbour, *et al*, 1984; Holton and Johnson, 1979). A high rate of sand mobility inhibits the growth of any but highly specialized plant species (Salisbury, 1952). Eldred and Maun (1982) pointed out that on sites with sand accretion there is (i) promotion of adventitious root growth; (ii) increase in moisture and lowering of soil temperature around the buried nodes, (iii) addition of nutrients by decomposing substrate; (iv) more nitrogen-fixing bacteria and mycorrhizal fungi associated with roots, and (v) genotypic differentiation.

Disraeli (1984) reported an increase in chlorophyll content per unit leaf area in buried plants *of Ammophila breviligulata* as compared to unburied plants in a naturally growing population Yuan *et al*, (1993) showed that artificial burial increased the net photosynthetic rate of *Ammophila breviligulata* and *Calamovilfa longifolia*. Experimental studies by Harris and Davy (1988) suggested that burial of *Elymus farctus* completely inhibited the net photosynthetic capacity of the buried assimilatory tissue but normal growth resumed quickly on re-exposure

Except for the three experimental studies mentioned above the physiological aspects of plant response to sand accretion have not been investigated any further Objectives of the present study are to examine the physiological and morphological responses of several annual (A), biennial (B), and perennial (P) dune plants to artificial burial in sand Plant parameters such as photosynthetic capacity, efficiency of net assimilation, leaf area, biomass and root/shoot ratio were measured

3.2 Material and Methods

3.2.1 Experimental Design

An area approximately 15 x 40 m was fenced off at Port Burwell Provincial Park on the shore of Lake Erie in May 1992 (Plate 3.1a). Six common dune species, *Agropyron psammophilum* (P), *Corispermum hyssopifolium* (A), *Panicum virgatum* (P), *Strophostyles helvola* (A), *Tusilago farfara* (P) and *Xanthium strumarium* (B) were chosen for this burial experiment Fifty plants of similar size and vigour of each of four species, *Corispermum hyssopifolium*, *Strophostyles helvola*, *Tusilago farfara* and *Xanthium strumarium*, were tagged. Five burial treatments, 0 (no burial--control), 0.33-buried to 1/3 of plant height (0.33 H), 0.66-buried to 2/3 of plant height (0.66 H), 1.00 complete burial of plant (1.00 H) and buried to complete plant height plus one-third of the plant height (1.33 H), were imposed on the 15th and 16th of June, 1992 For buried and control plants, white plastic drainage pipes (12 cm diameter) were placed around the plants and then filled to the appropriate depth by using sand from the same habitat (Plate 3.2b).

Plate 3.1 a Fenced area in which the burial experiments were conducted at Port Burwell Provincial Park.

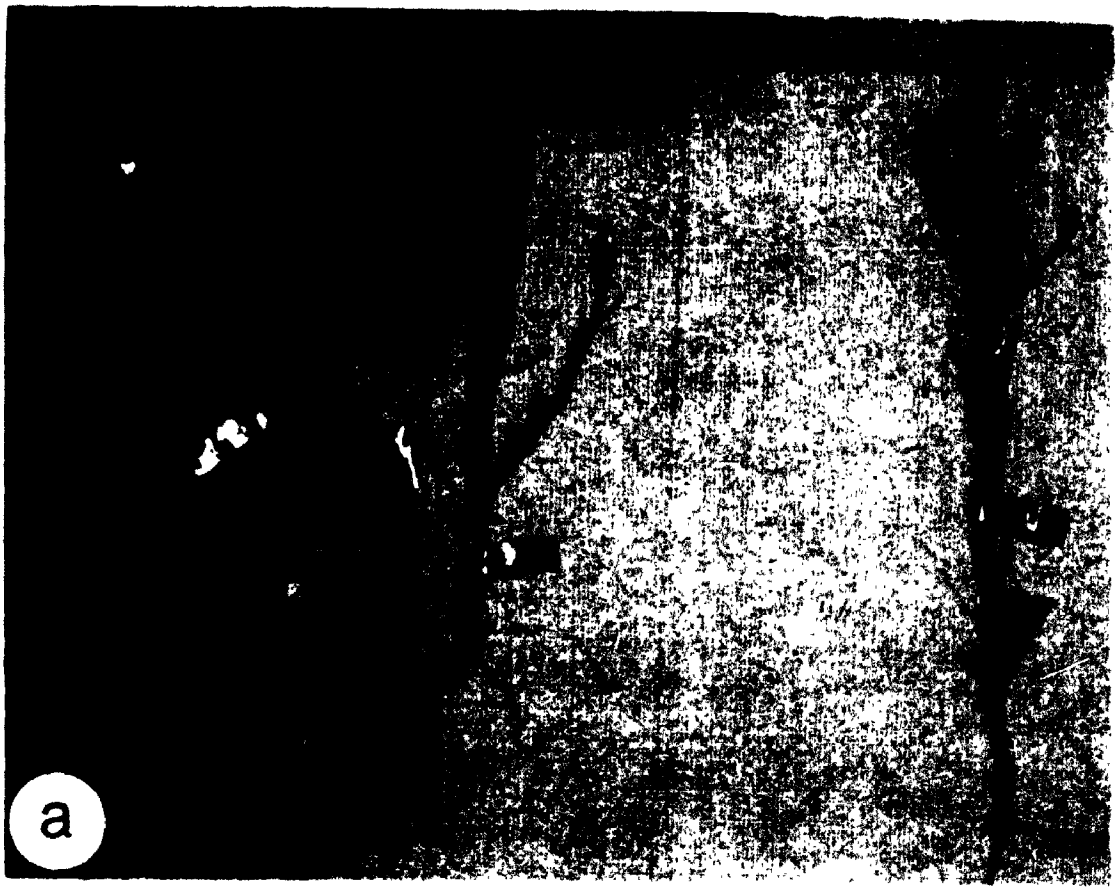
b Wooden frame used for burial of *Panicum virgatum* plants to 20 cm depth


a


b

Plate 3.2 a. Photograph showing harvested plants of *Corispermum hyssopifolium* (from left, control, 0 33 H and 0.66 H).

b Photograph showing buried plants of *Xanthium strumarium* (from left, 0 33 H and 0 66 H)


a


b

For the burial of the two perennial grasses. *Agropyron psammophilum* and *Panicum virgatum* wooden frames (0 5 x 0 5 cm) were used (Plate 3 1b). In October, 1992 the wooden frames were stacked to appropriate heights on naturally growing populations of the two grasses Five burial treatments, 0 (control), 20, 40, 60 and 80 cm deep sand, were then superimposed on plants within each frame. There were 8 replications for each treatment (plates 3 1b and 3.3). The emergence of plants from burial treatments was recorded in early spring, 1993. The plants of *Panicum virgatum* that were buried at 20 and 40 cm began emerging from the sand surface by the middle to the end of May, 1993 Similarly, the plants of *Agropyron psammophilum* buried to 20 and 40 cm both emerged at the end of May.

The plants, *Corispermum hyssopifolium* (A), *Strophostyles helvola* (A), *Tusilago farfara* (P) and *Xanthium strumarium* (B) were allowed to grow for approximately 3 weeks after burial treatments were imposed, then net photosynthetic measurements were taken For *Agropyron psammophilum* (P) and *Panicum virgatum* (P) measurements of carbon dioxide exchange rate (CER) were taken in summer of 1993 after the emergence of plants. The first set of readings was taken 308 days after the plants were buried In both species, the plants buried to 60 and 80 cm depths did not emerge at all, and therefore these two treatments were not included in data analysis.

3.2.2 Measurement of CO_2 Gas Exchange

CO_2 exchange rates were measured in a closed system using a LICOR-6200 (Lincoln, NE, USA) Infra Red CO_2 analyser. The LI-6200 consists of 3 main components, namely the (i) leaf chamber, where air temperature, leaf temperature and humidity measurements are made; (ii) LI-6250, which measures CO_2 concentration and flow rate, and (iii) a control console. The pump in the LI-6250 circulates air from the chamber to the analyzer, where CO_2 concentration is measured, and returns it to the chamber The air flow through the LI-6250 can be directed through soda lime to remove CO_2 for calibrating the system The flow valve is used to force some portion of the flow through a tube containing magnesium chlorate, a desiccant, which dries the air. This control feature is used to help maintain a steady humidity in the chamber

Plate 3.3 a The 40 cm deep burial treatment of *Agropyron psammophilum* at Port Burwell Provincial Park Two emerged shoots can be seen at the sand surface.

b. The 80 cm deep burial treatment. The shoot visible on the surface has germinated from seed and is not the original buried plant


a


b

during a measurement The flow rate of the air passing through the desiccant is measured by a flow meter The net exchange of CO_2 between a leaf and the atmosphere was readily measured using the LI-6200 by enclosing the leaf in a closed chamber, and monitoring the rate at which the CO_2 concentration in the air changes over a fairly short time interval (typically 10-20 seconds)

The net CO_2 gas exchange rate is then calculated using the rate of change of CO_2 concentration and other factors, such as the area of leaf that was enclosed in the chamber, the volume of the enclosed chamber, temperature, and pressure The measurements were done on attached, fully expanded second leaves under clear-sky conditions between 10 A M and 2 P M For each species the measurements were taken randomly on the same day under similar light and temperature conditions Three readings were taken each time, and this was repeated three times at approximately three week intervals The CO_2 uptake readings, is a measure of the photosynthetic capacity of the plant's photosynthetic process Photosynthetic capacity is defined as the light saturated rate of CO_2 uptake

3.2.3 Measurement of F_v / F_m

At the completion of the net photosynthetic readings (same day or the following day), the second fully expanded leaf from each plant was cut, and immediately placed into a flask containing water, it was then taken to the laboratory, where it was dark adapted for 45-60 minutes at room temperature and F_v/F_m was measured using the PSM (plant stress meter) chlorophyll fluorometer (Biomonitor S C I ab, Umeå, Sweden) The fluorescence parameter, F_v/F_m is correlated with the photochemical efficiency of photosystem II, as is the quantum yield of oxygen.

3.2.4 Measurement of Leaf Area and Biomass

After the completion of the CO_2 exchange rate and the photochemical efficiency measurements, the plants were harvested The annuals, biennials and the perennial, *Tusilago farfara* were harvested on August 19 and 20, 1992 Before harvest each plant was tagged and the stem was marked with a permanent marker at the sand

surface The drainage pipes enclosing the plants were lifted, the sand was removed to the pre-burial level, and plant stem was marked at the preburial point with a permanent marker An area of 30 cm radius around each plant was dug to a depth of 20-30 cm (depending on the root length of the species) and the plant was carefully removed from the ground. The roots were washed, the plants were tagged and then placed between moist newspaper in a trough and taken to the laboratory for morphological measurements (Plate 3.3a) Leaf area was measured using a leaf-area meter (LI-3000), and each plant was separated into leaves, above-ground stem, below-ground stem, and roots Each fraction were then placed in separate paper bags, dried at 70°C for 48 hours, and weighed. Root/shoot ratios were also calculated.

The perennial grasses were excavated between August 30th and september the 3rd, 1993 to study the effects of burial on the mode of regeneration and growth. First the surface level was marked on the stem with a permanent marker, then the wooden frames were removed carefully so that no roots or other plant parts were damaged. The sand around the plants was then removed to a depth of 30 cm below the original pre-burial sand surface The plants were removed from the ground with a shovel and placed in paper bags, labelled and taken to the laboratory, where they were kept in a cool room at 4°C until further morphological measurements were made.

A sample of 4 plants was randomly chosen from each replication and measurements were taken on leaf area, dry biomass and root/shoot ratio.

3.2.5 Data analysis

Analysis of data was done using the general linear model (GLM) procedures in the SAS statistical package The mean net photosynthetic rate (CER), photochemical efficiency (F_v/F_m), leaf area , biomass and root/shoot ratio were calculated and their values in each treatment were compared following one way ANOVA and Turkey's multiple range test

3.3 Results

3.3.1 Effects of Partial Sand Burial on CO_2 Gas Exchange (CER) on Some Common Dune Species.

Partial sand burial of dune plants under field conditions had different responses between species but the overall trend was quite similar even between annuals and perennials In *Tussilago farfara* the CER was significantly lower in the 0.66 H plants than in the control plants throughout the 54 days of burial (Fig 3 1) The CER for the 0.33 H burial showed an increase over the control after 20 days and was significantly higher than control after 42 days However, it later decreased, and became significantly lower than control by the 54th day after burial

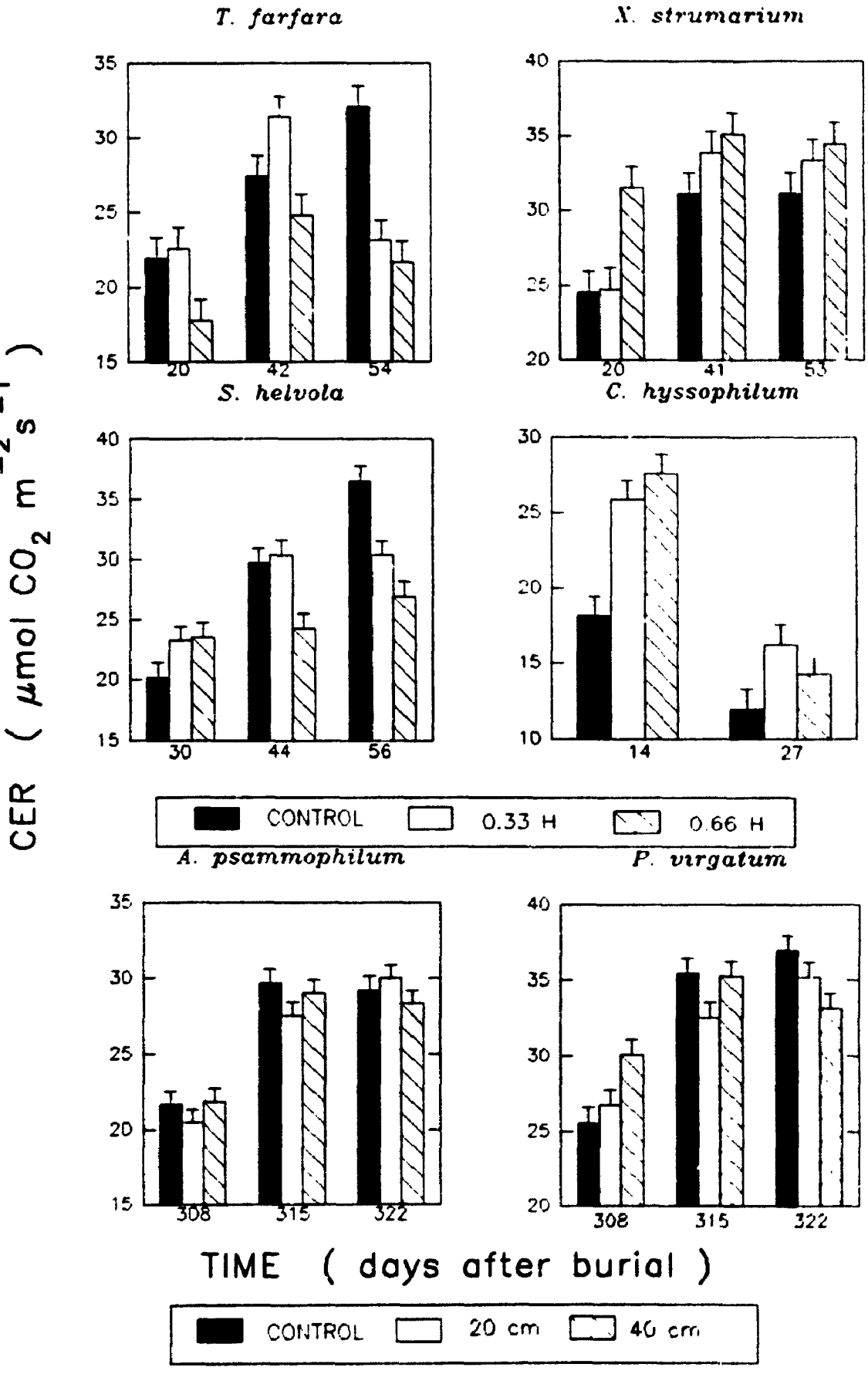
In *Xanthium strumarium* a significant increase of the CER was seen in the 0 66 H plants within 20 days, while the 0 33 H plants did not show a significant increase in the CER until 41 days after the burial treatment There was a significant difference between the control and the buried plants even 53 days after burial (Fig 3 1).

Strophostyles helvola exhibited a significant increase in the CER in both the 0.33 H and the 0 66 H plants over the control after 30 days of burial Following 44 days of burial, the 0 66 H treatment had significantly lower CER than the control and 0.33 H plants At the third reading (56 days after burial), 0.66 H plants were significantly lower than 0.33 H and both were significantly lower than the control plants (Fig 3 1)

In *Corispermum hyssopifolium* there was a significant increase in the CER of buried plants within about 14 days after burial The 0 66 H plants were also significantly higher in their CER as compared to the 0 33 H plants The buried plants indicated a significant increase in the CER at 27 days after burial as compared to control, but the 0.66 H plants were significantly lower than the 0.33 H plants (Fig 3.1)

In *Agropyron psammophilum* and *Panicum virgatum*, for all three sets of readings the CER was significantly higher for both the burial treatments (20 and 40 cm) as compared to the control. In *Agropyron psammophilum* the CER was

Figure 3.1 Mean (± S E) carbon dioxide exchange rate of *Agropyron psammophilum*, *Corispermum hyssopifolium*, *Panicum virgatum*, *Strophostyles helvola*, *Tusilago farfara* and *Xanthium strumarium* on different dates at 0 00 H, 0 33 H and 0 66 H burials


T. farfara
X. strumarium
S. helvola
C. hyssophilum
A. psammophilum
P. virgatum
CER (μmol CO2 m-2 s-1)
CONTROL
0.33 H
0.66 H
TIME (days after burial)
CONTROL
20 cm
40 cm

significantly higher for the 40 cm than the 20 cm burial treatment after 315 days However, the 40 cm burial treatment showed no difference in CER compared to control and the 20 cm burial treatment. In *Panicum virgatum*, the CER for plants buried at 40 cm was significantly higher at the first (308 days) and second (315 days) sets of readings but was significantly lower in the third reading (322 days after burial) (Fig. 3 1)

3.3.2 Effect of Partial Burial in sand on the Photochemical Efficiency of Some Common Dune Species

The photchemical efficiency (F_v/F_m) measurements of buried plants of *Tusilago farfara* and *Xanthium strumarium* were not significantly different from control (Fig 3 2) In *Strophostyles helvola* there was a significant increase in the F_v/F_m of buried plants over control In *Corispermum hyssopifolium* buried plants had a significantly higher F_v/F_m than control, however, plants in the 0.66 H treatment had slightly lower values than the 0.33 H plants. (Fig. 3.2).

3.3.3 Effect of Partial Sand Burial on Plant Morphology of Some Common Dune Species

The leaf area of buried plants was greater than control in all three annual species For *Corispermum hyssopifolium* the standard error was high and the differences in leaf area were not significant. In *Strophostyles helvola* there was a significant increase in the leaf area of buried plants in the 0.33 H treatment but the 0 66 H treatment was not significantly greater than control. The buried plants of *Tusilago farfara* and *Xanthium strumarium* in both treatments had significantly greater leaf area than control (Fig 3 3).

The dry biomass of buried plants was higher than control in all four species. In *Corispermum hyssopifolium* the 0.66 H plants produced significantly higher biomass than control, but the 0 33 H plants were not significantly different than control or the 0 66 H plants In *Strophostyles helvola, Xanthium strumarium* and *Tusilago farfara* although there were no differences between the two burial depths, each one of them

Figure 3.2 Mean (±S.E) photchemical efficiency of *Corispermum hyssopifolium*, *Strophostyles helvola* *Tusilago farfara* and *Xanthium strumarium* buried to depths of 0% (0 00 H), 33% (0.33 H) and 66% (0 66 H) of their height

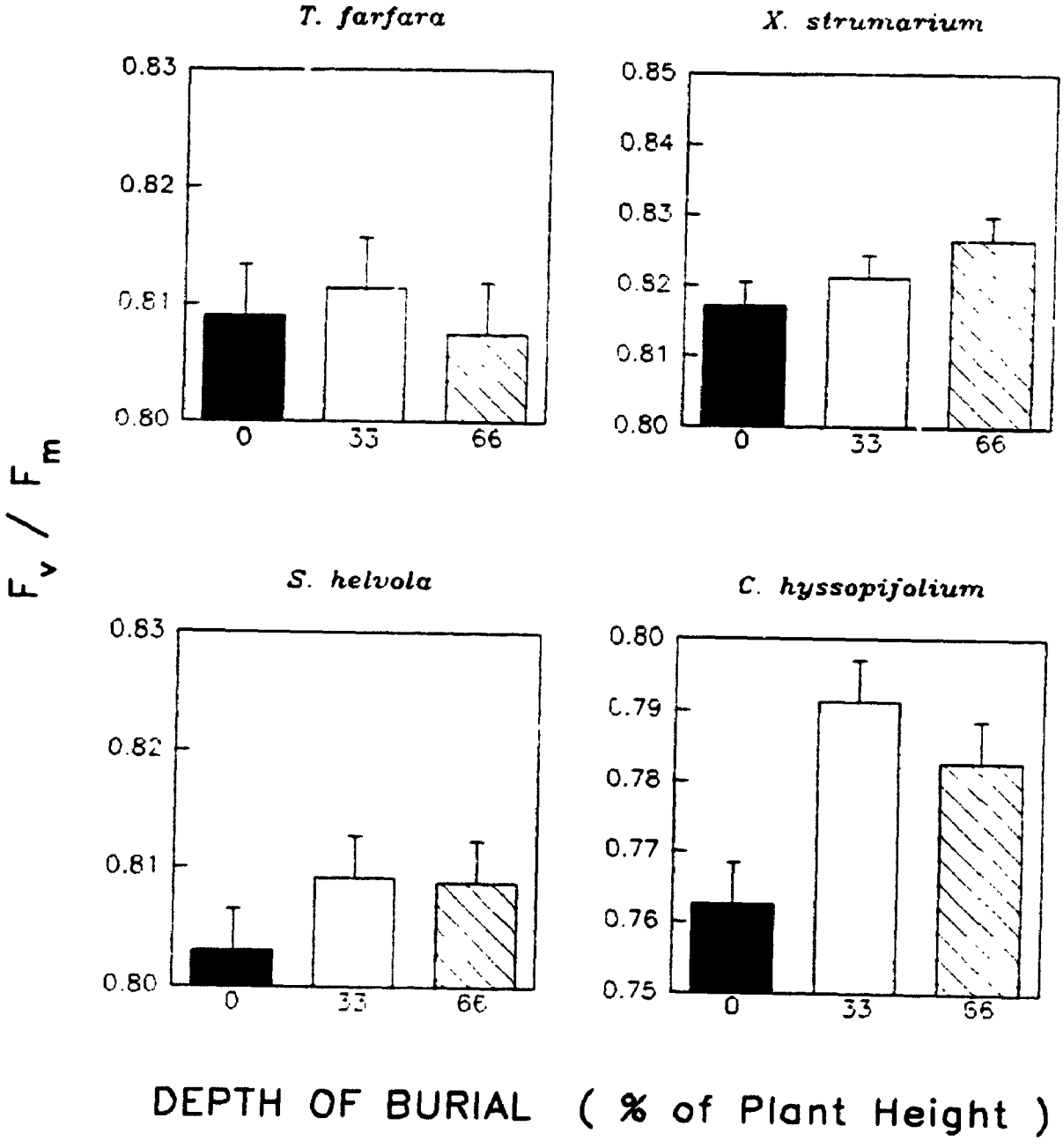

T. farfara
X. strumarium
S. helvola
C. hyssopifolium
F_v / F_m
0.83
0.82
0.81
0.80
0.85
0.84
0.83
0.82
0.81
0.80
0.79
0.78
0.77
0.76
0.75
0
33
66
DEPTH OF BURIAL (% of Plant Height)

Figure 3.3 Mean (± S.E) leaf area of *Corispermum hyssopifolium*, *Strophostyles helvola*, *Tusilago farfara* and *Xanthium strumarium* plants buried to depths of 0% (0.00 H), 33% (0.33 H) and 66% (0 66 H) of their height.

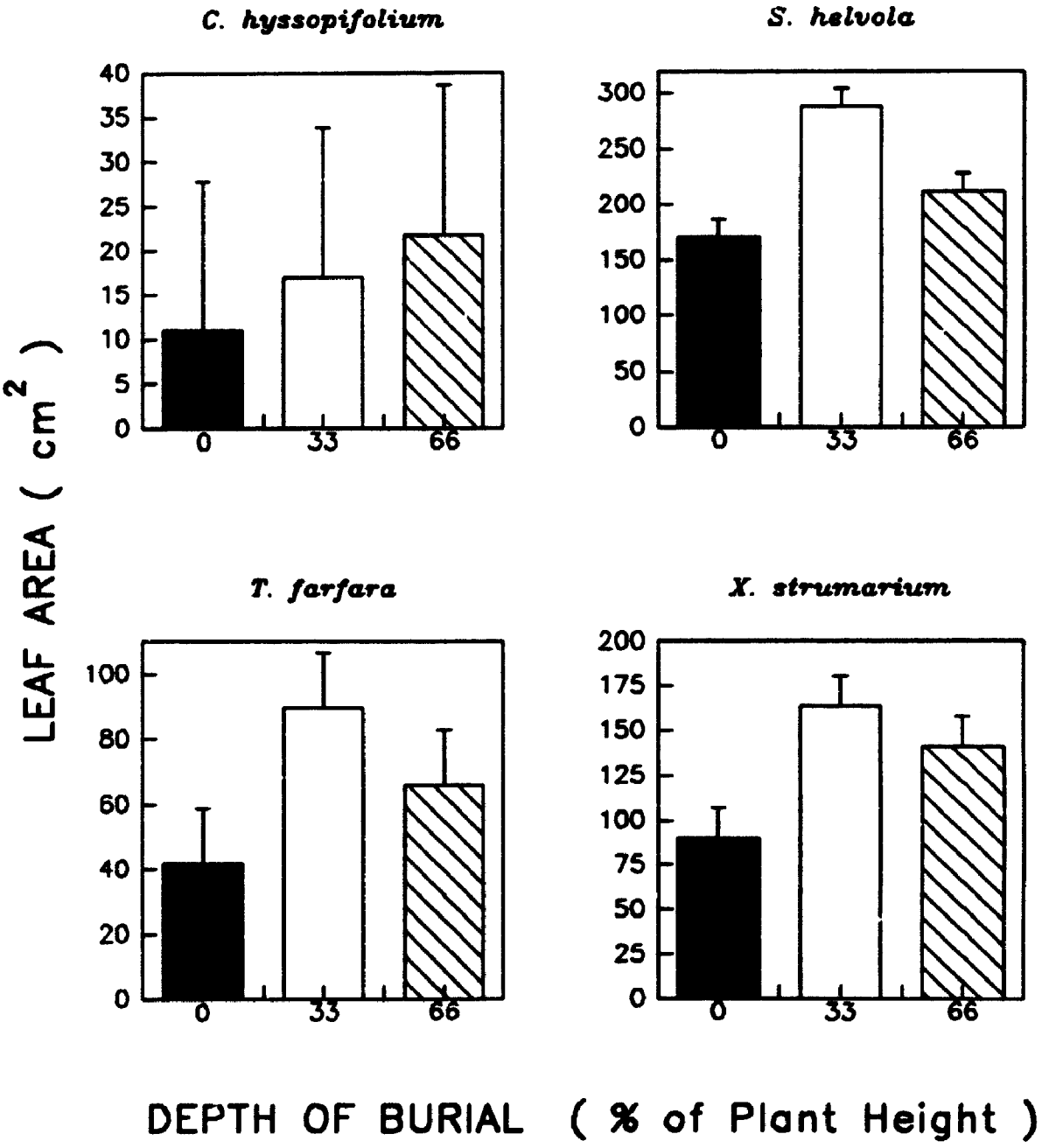

C. hyssopifolium
S. helvola
T. farfara
X. strumarium
LEAF AREA (cm 2)
0
33
66
DEPTH OF BURIAL (% of Plant Height)

Figure 3.4 Mean (± S.E.) biomass of *Corispermum hyssopifolium*, *Strophostyles helvola*, *Tusilago farfara* and *Xanthium strumarium* buried to depths of 0% (0 00 H), 33% (0 33 H) and 66% (0 66 H) of their height

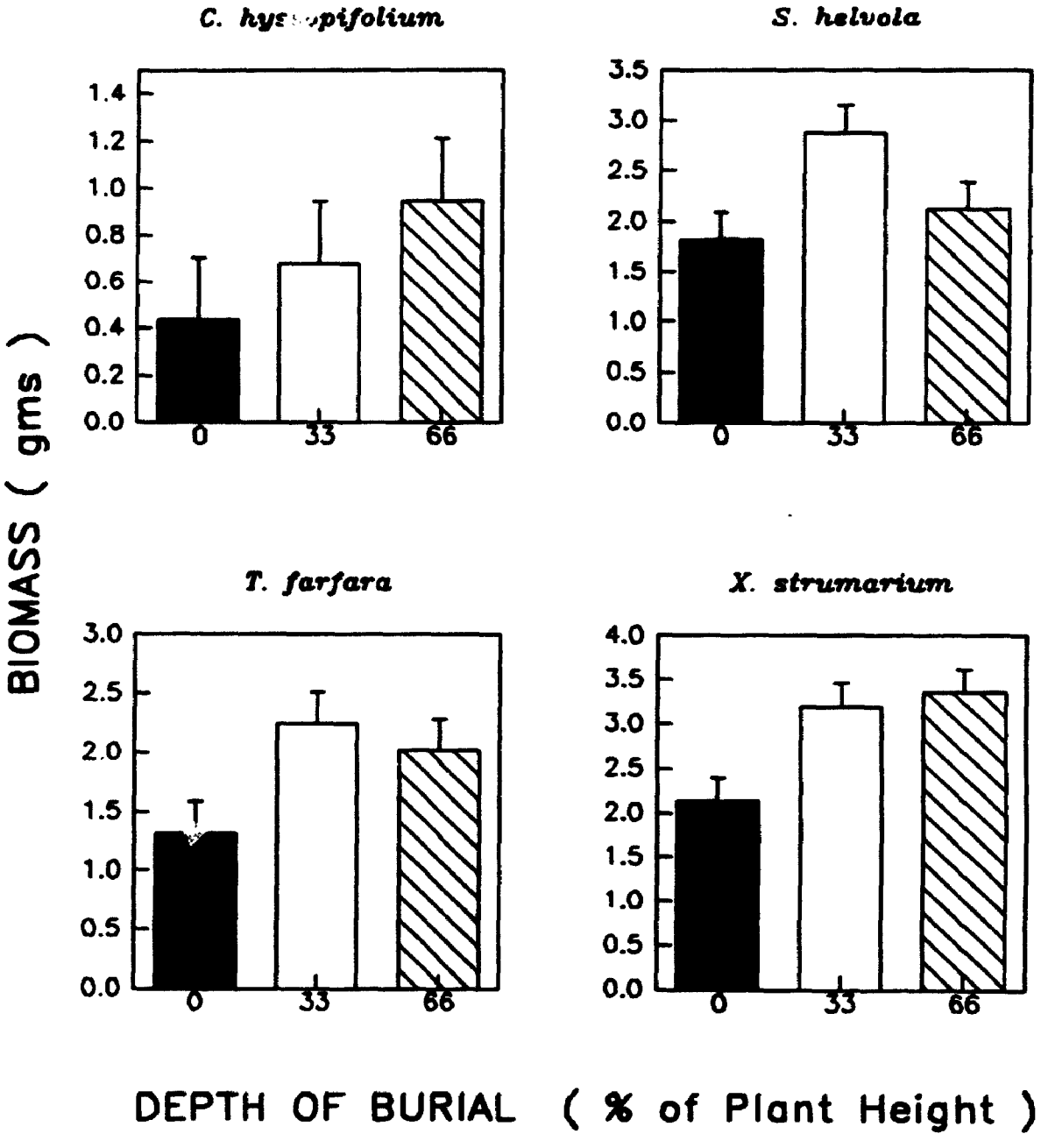
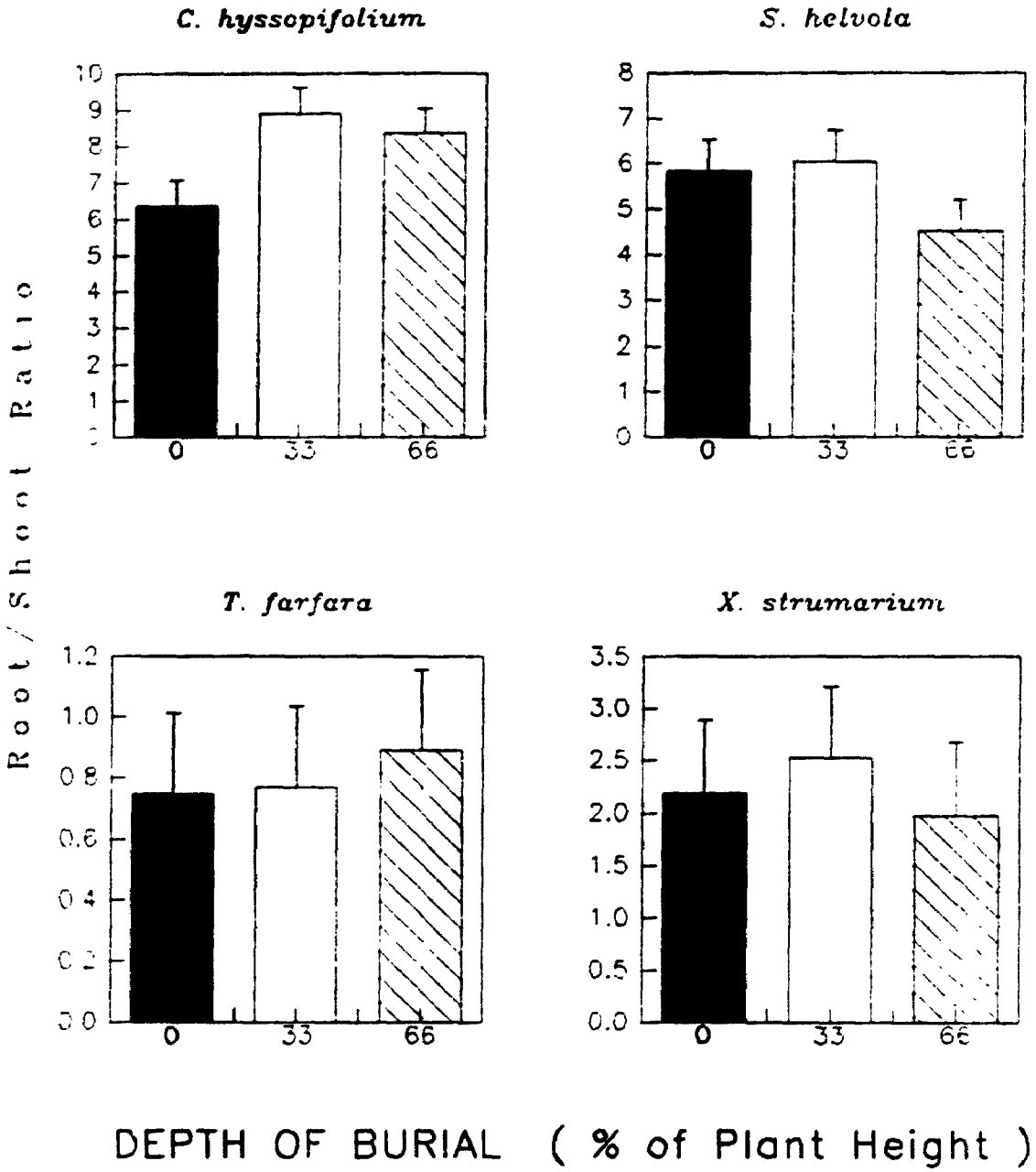

C. hyssopifolium
S. helvola
T. farfara
X. strumarium
BIOMASS (gms)
0
33
66
DEPTH OF BURIAL (% of Plant Height)

Figure 3.5 Mean (± S.E.) root/shoot ratio of *Corispermum hyssopifolium*, *Strophostyles helvola*, *Tusilago farfara* and *Xanthium strumarium* buried to depths of 0% (0.00 H), 33% (0.33 H) and 66% (0.66 H) of their height


C. hyssopifolium
S. helvola
T. farfara
X. strumarium
Root / Shoot Ratio
0
33
66
DEPTH OF BURIAL (% of Plant Height)

produced significantly higher biomass than control (Fig. 3.4).

Except for *Corispermum hyssopifolium* there were no differences in the root/shoot ratio between control and the two burial treatments. In *Corispermum hyssopifolium* root/shoot ratio of buried plants was significantly higher than control (Fig. 3.5). At harvest it was noted that new roots and root hairs had developed on the nodes of the buried stems.

3.4 Discussion

From this burial experiment it was clear that although there were significant differences between species, all dune species tested (*Tusilago farfara*, *Xanthium strumarium*, *Strophostyles helvola*, *Corispermum hyssopifolium*, *Agropyron psammophilum*, and *Panicum virgatum*) exhibited stimulated growth following burial in sand. This was shown by an increase in the net CO_2 uptake, leaf area, and biomass per plant. Similar responses were observed by Yuan *et al.*, (1993) in *Ammophila breviligulata* and *Calamovilfa longifolia* plants. However, in general the stimulation in net CO_2 uptake did not last long. The stimulation occurred only during the first two to three weeks after burial and then it levelled off or started to decline. This could be due to the onset of senescence of leaves which is usually manifested by yellowing and breakdown of chlorophyll and hence a decrease in rate photosynthesis. The decrease in photosynthetic rate before senescence could probably be due to reduced demand for photosynthate. In most plants this decrease in photosynthesis is soon followed by increased respiratory activities (Bidwell, 1974). The significant increase in the leaf area was probably of greatest benefit to the plant because it is translated into a significant increase in biomass.

In natural dune systems, the foredune plants are regularly inundated by fresh sand. Following burial, a plant spends all its energy to emerge above the sand surface Harris and Davy (1986). Once it has recovered from this episode, the plant is able to utilize the resources contained in the inundated sand and expand into the new soil surface area created by the newly deposited sand. Between species differences became

evident during recovery In general, the stimulation in growth of annuals started sooner than the perennials They also did not tolerate high amounts of sand burial For example most of the annual plants did better when buried at 33% of their height than that of the two perennial grasses. *Agropyron psammophilum* and *Panicum virgatum*.

The positive effects of burial were much more pronounced in the two perennial grasses *Agropyron psammophilum* and *Panicum virgatum*. Both species are rhizomatous and produced new tillers after emergence from the sand deposits. The new growth is dependent on energy stored in the roots and rhizomes However, the maximum burial depth from which they emerged was 40 cm, which shows that they are not as efficient as *Calamovilfa longifolia*, which can withstand 60 cm of sand burial per year and *Ammophila breviligulata* which can withstand up to 100 cm of burial in sand per year (Maun, 1985; Maun and Lapierre, 1984).

The photochemical efficiency (F_v/F_m) measurement was a very important indicator of the state of photosystem II and measured the photochemical efficiency of the process. The photochemical efficiency increased only slightly in all treated plants except in the case of *Strophostyles helvola* and *Corispermum hyssopifolium* in which there was a significant increase Thus, the increase in the photosynthetic capacity (net CO_2 uptake) was related to an increase in the F_v/F_m.

The analysis of the leaf area clearly shows that buried plants had greater leaf area This agrees with the results of Disraeli (1984), who reported significantly higher leaf area for buried plants Yuan *et al.*, (1993) also reported a slight increase in the leaf area According to Olson (1958b) larger leaf area not only enhances leaf area index, but also increases total evapotranspiration. He argued that since buried plants had better access to moisture conditions than unburied plants, increased evapotranspiration rate increased water and nutrient absorption in buried plants, and thus resulted in improved growth.

From this study I found that there was a significant increase in plant biomass of buried plants as compared to control probably due to the compensatory ability of plants in response to the pressure imposed by sand burial. Another reason for the stimulated growth resulting in increased biomass may be the result of mycorrhizal association which enhances the uptake of nutrients, especially phosphorus in coastal

dune species (Koske and Polson, 1984, Koske *et al*, 1975) My work clearly showed that the amount of sand burial from which a plant survived, was specific to the species, and although moderate amounts of sand burial promoted growth, excessive and prolonged burial decreased the vigour or even prevented the emergence of plants from burial depths The presence of new roots and root hairs in the buried part of stems not only provided nutrients, moisture and extra space for expansion of roots but also firmly anchored the plants in the sandy substrate

In conclusion, field experiments at port Burwell Provincial Park have shown that buried plants of the six species tested exhibited higher net photosynthesis, F_v/F_m, leaf area and dry biomass as compared to control The trend was evident even though many environmental factors, such as fluctuations in light intensity, temperature, wind, velocity etc could not be controlled under field conditions

CHAPTER FOUR

ECOPHYSIOLOGICAL RESPONSE TO SAND ACCRETION IN CONTROLLED CONDITIONS

4.1 Introduction

The investigations reported in chapter 3 of this thesis, included a study of the physiological and morphological responses of several dune species to sand burial under field conditions where plant density, interspecific competition, diurnal changes in the physical environment and other biotic factors could not be controlled. In this study we studied the responses of several common dune species, to various depths of sand burial under greenhouse conditions and in a walk-in growth chamber where light intensity, temperature and relative humidity were controlled.

Besides the physiological and morphological analysis that were described in chapter 3, we have examined total chlorophyll content, chlorophyll a/b ratios and leaf thickness of the different dune species used in these experiments. Through these experiments we hope to have a better understanding of the physiological and morphological responses of eight sand dune species to sand accretion under different temperature and light intensity regimes

4.2 Methods and Materials

4.2.1 Experimental Design

(i) GREENHOUSE: A greenhouse experiment was initiated to test the effect of sand burial on individual sand dune plants. The seeds of 8 dune species, *Agropyron psammophilum*, *Cakile edentula*, *Corispermum hyssopifolium*, *Elymus canadensis*, *Oenothera biennis*, *Panicum virgatum*, *Strophostyles helvola*, and *Xanthium strumarium* collected in September, 1991, were cleaned, and stored in a cold room at 5°C and 40% RH. Four lots of 50 seeds of each species were placed in separate Petri

dishes, underlaid with two sheets of moistened Whatman No 1 filter paper and set to germinate in an incubator maintained at 25°C day (14 hr) and 20°C night (10 hr) temperatures *Corispermum hyssopifolium* received cold treatment of below freezing temperatures (- 4°C) for approximately a month prior to germination and *Panicum virgatum* was given cold treatment of 0°C for two weeks prior to germination. Germinated seeds were planted on a sand tray and left for 2-3 weeks in a greenhouse at 24/10°C day/night temperatures Seedlings of similar shape and size were chosen for each species and transplanted into 6" plastic pots. The plants were watered every other day, and nutrient solution, containing N P K (20:20:20) was added once a week to reduce the possibility of nutrient deprivation due to sand burial. The burial treatments were applied 10-12 weeks after transplanting to individual pots.

The eight dune species comprising four annuals, two biennials, and two perennials were buried to different depths. no burial (0 0-control); burial to 1/3 of plant height (0.33 H), burial to 2/3 of plant height (0.66 H); burial to full plant height (1 00 H); and burial to 1.33 times the plant height (1.33 H)

In most species, the 1.00 H and 1.33 H plants did not emerge from the burial treatments However, some seedlings (1-3 replicates) of *Cakile edentula, Panicum virgatum, and Elymus canadensis* emerged from these depths. Therefore, the data from 1.00 H and the 1.33 H emerged plants were not included in the analysis.

(ii) GROWTH CHAMBER: The effect of burial was also examined under controlled light and temperature regimes in a walk-in growth chamber at the Agriculture Canada Research Centre, London, Ontario (Plate 4 1) Individually potted plants (12 weeks old) of six common dune species--*Agropyron psammophilum, Cirsium pitcheri, Elymus canadensis, Oenothera biennis, Panicum virgatum,* and *Strophostyles helvola* were transferred to the growth chamber on December 28, 1992. These plants had been germinated and transferred to individual pots following similar procedures mentioned in the greenhouse experiment There were six replicates and three burial treatments according to plant height (H) above the sand surface, 0 0 H (no burial--control), 0.33 H (one-third burial) and 0.66 H (two-thirds burial)

In growth chamber, the carbon dioxide gas exchange rate (CER) was measured under the following temperature and light regimes. (a) at constant temperature (25°C)

Plate 4.1 Sand burial experiment with dunes plants in the walk-in growth chamber at the research centre of Agriculture Canada, London


a


b


c

and varying light intensities (500, 1000, 1500, and 2000 μmol $m^{-2}s^{-1}$); (b) at constant light intensity (1500 μmol $m^{-2}s^{-1}$) and varying temperatures (20, 25, 30, and 35°C); and (c) at constant light intensity (1500 μmol $m^{-2}s^{-1}$) and constant temperature (25°C) over time at 13, 20 and 27 days after burial. The readings were taken randomly for various treatments of light intensity and temperature were recorded at two day intervals to give the plants enough time to acclimatize to the changed light or temperature regimes.

4.2.2 Measurements of CO_2 Gas Exchange

(i) GREENHOUSE: CO_2 gas exchange was measured using the LI-6200 as was done in the field experiment (chapter 3). In the greenhouse the daytime temperature was set at 24°C, but factors like light intensity and relative humidity were difficult to control. To minimize the variations in light intensity the readings were taken between 10.00 AM and 2.00 PM on bright sunny days. Three readings were taken each time randomly on each of the treatments, and this was repeated three times at three-week intervals over the course of the experiment.

(ii) GROWTH CHAMBER: Two weeks after the plants were given burial treatments, three experiments involving net CO_2 gas exchange were measured. **Experiment I**- constant light (1500 μmol $m^{-2}s^{-1}$) and constant temperature (25°C) over time, in which CER measurements were taken on a weekly basis for three weeks; **Experiment II**- constant light (1500 μmol $m^{-2}s^{-1}$) and varying temperatures (20, 25, 30, and 35°C), and **Experiment III**- constant temperature (25°C) and varying light intensities (500, 1000, 1500 and 2000 μmol $m^{-2}s^{-1}$). Three readings were taken for each experiment at 7 day intervals. Measurements at various light intensities and temperatures were taken

4.2.3 Measurement of Photochemical Efficiency

(i) GREENHOUSE: F_v/F_m was measured on the 2nd, 3rd and 4th fully expanded attached leaves of each plant. Three sets of readings were taken one on each leaf on the same day or a day after the net photosynthetic readings were taken. The plants were dark adapted for one hour before the measurements were taken randomly.

(ii) GROWTH CHAMBER:- F_v/F_m measurements were taken also on the 2nd, 3rd and 4th fully expanded attached leaves of each plant on the same day after recording CO_2 uptake Plants were dark adapted for about one hour before taking the measurements

4.2.4 Total Chlorophyll and Chlorophyll a/b Ratio

GROWTH CHAMBER When all the readings for the experiment in the growth chamber were completed, the 2nd fully expanded leaf was removed from each plant and analyzed for total chlorophyll content and chlorophyll a/b ratio. The leaves were washed in distilled water and ground singly with 80% acetone in a mortar and pestle The mortar was placed in a bucket of ice to prevent proteins in the leaves from denaturing A small amount of purified sand was added where necessary to aid in the grinding of the leaves. The extract was then poured into a test tube Any remaining extract was also rinsed into the test tube by washing the mortar and pestle with a small amount of 80% acetone. The test tube was then corked, and wrapped with aluminium foil, to avoid degradation of pigments and kept in refrigerator until all the extractions were completed. The test tubes were shaken and a portion poured into a small centrifuge tube and centrifuged at 12000 g for 30 seconds in an Eppendorf centrifuge-5414. Absorbance of the supernatant at 663 and 645 nm was determined using a spectrophotometer (Shimadzu, uv-visible recording spectrophotometer, UV-160) The chlorophyll a/b ratio and the total chlorophyll contents were calculated using the equations described in Arnon, 1949

4.2.5 Measurement of Leaf Area, Biomass and Root/Shoot Ratio

(i) GREENHOUSE:- Plants used in the greenhouse experiment were harvested from August 26 till September 8, 1992. The techniques used in the harvest of plants was similar to that described in chapter 3, for the field investigations Leaf area, dry biomass and the root/shoot ratios were calculated and analyzed, as described in chapter 3 of this thesis

(ii) GROWTH CHAMBER - The plants used in the growth chamber

experiment were harvested between January 28 and February 9, 1993. The procedures used in harvesting of these plants were again similar to the ones used in the greenhouse and field experiments. Leaf area, dry biomass and the root/shoot ratios were calculated and analyzed as mentioned in chapter 3, of this thesis.

4.2.6 Data analysis

Analyses of data obtained from the greenhouse and growth chamber experiments were done by using the General linear model (GLM) procedures in the SAS statistical package. The mean net photosynthetic rate (CER), photochemical efficiency (F_v/F_m), leaf area, biomass, root/shoot ratio, chlorophyll a/b ratio, total chlorophyll content and leaf thickness were calculated

4.3 Results

4.3.1 Effect of Sand Burial on Carbon dioxide gas exchange under controlled conditions in some common dune plants

(i) GREENHOUSE. Results of the greenhouse experiments were generally similar in trend as those of the field investigations which were reported in chapter 3 of this thesis

In *Agropyron psammophilum* the carbon dioxide exchange rate (CER) did not differ until the third reading (56 days after burial), following which there was a significant increase in the CER of the 0.33 H and 0.66 H buried plants over the control. The CER of the 0.66 H buried plants was also significantly higher than that of 0.33 H buried plants (Fig. 4.1) .

Oenothera biennis showed a significant increase over the control plants in the CER readings of the 0.33 H and 0.66 H buried plants at the first reading (21 days after burial) Similarly, the 0.66 H buried plants were also significantly higher in its CER than the 0 33 H plants (Fig 4.1 and Plate 4.2a). The CER readings levelled off at the second and third readings 42 and 70 days after burial, respectively.

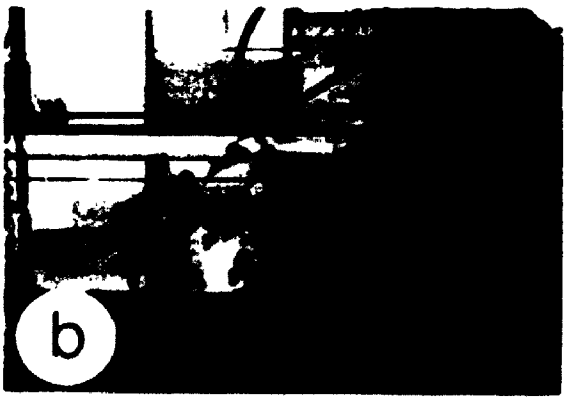
In *Strophostyles helvola* there was a significant increase in the CER of the 0.33

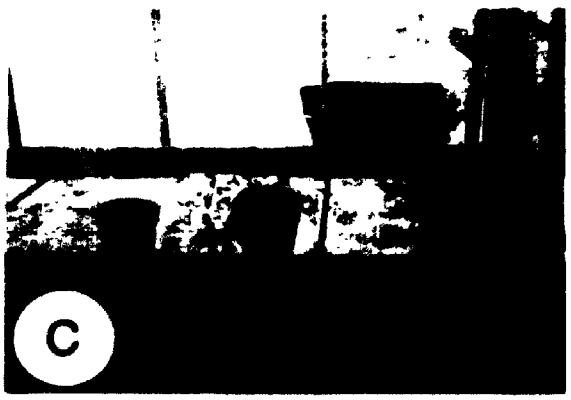
Plate 4.2 a The greenhouse burial experiment with *Oenothera biennis*, control plant at the left (not bolting), followed by 0.33 H and 0.66 H plants which had bolted. On the extreme right is the 1.00 H treatment in which the buried plants did not emerge

b *Xanthium strumarium* showing control plant on the left, the 0.33 H plant in the centre and 0.66 H plant on the right

Cakile edentula, control plant on the left, the 0.33 H plant in the centre and the 0.66 H plant on the right. The control plant had flowered and senesced earlier than the treated plants


a


b


c

H plants over the control plants, 14 days after burial but there was no difference between the control plants and the 0 66 H plants At the second reading (28 days after burial), there was no difference between control and 0 33 H plants The 0 66 H plants had a significantly greater CER than control and 0 33 H plants (Fig 4 1)

Panicum virgatum showed a significant increase in the CER of 0 33 H buried treatment as compared to control at the first reading, (25 days after burial). Similar increase was not observed in 0.66 H plants on this date At the second reading (48 days after burial), the CER of the 0 33 H plants were still significantly higher than control plants, but the 0.66 H plants were significantly lower in their CER than control At the third reading, 56 days after burial, both 0 33 H and 0 66 H were significantly higher in their CER than the control plants (Fig 4 1 and Plate 4 3a)

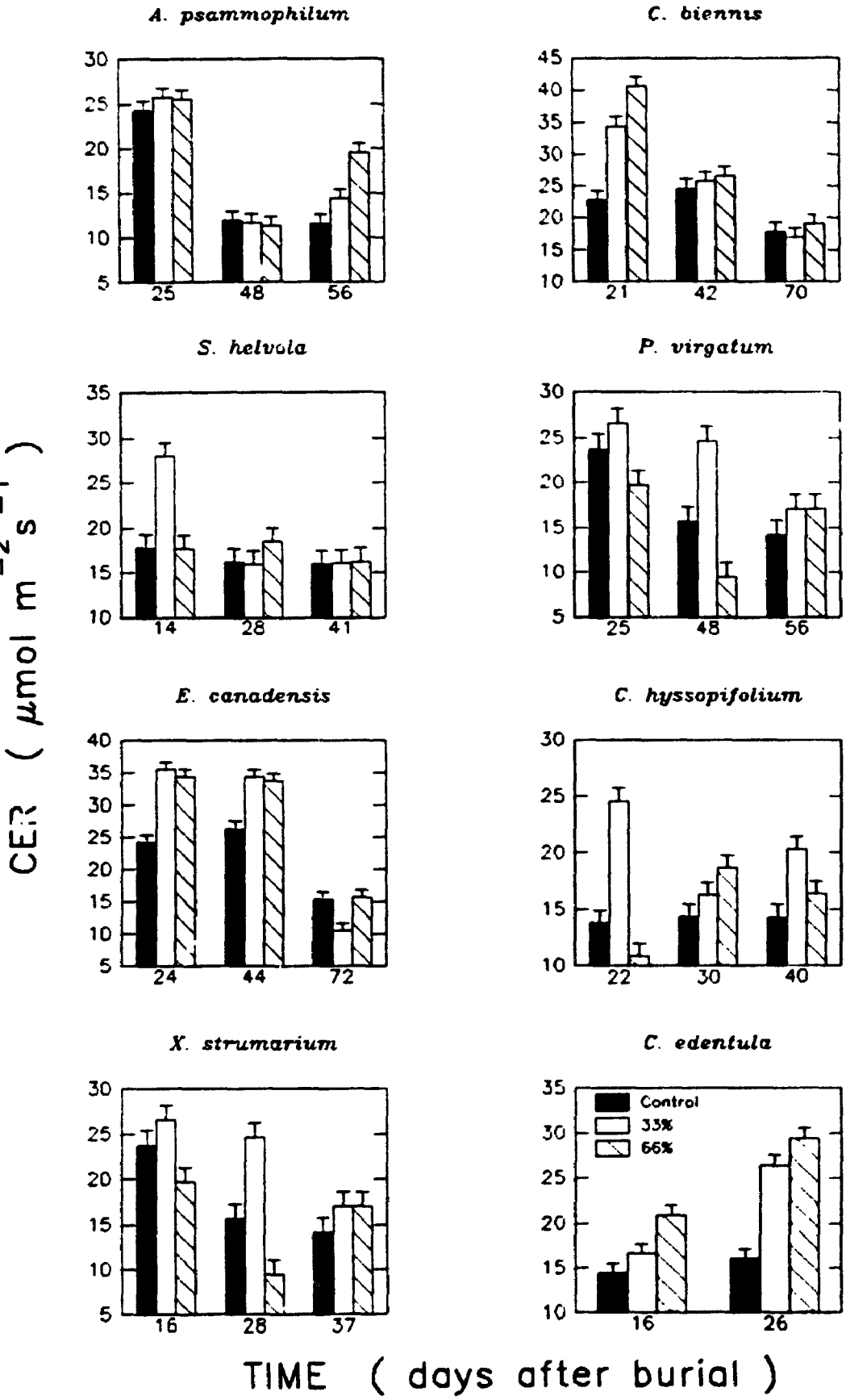
In *Elymus canadensis* 0.33 H and 0 66 H plants had significantly higher CER than the control plants both at 24 and 44 days after burial After 72 days of burial, the control and 0 66 H plants had similar CER, but 0 33 H plants were significantly lower in their CER values than control (Fig 4 1)

In the case of *Corispermum hyssopifolium* the 0 33 H plants showed a significant increase in the CER over the control plants at the first reading (22 days after burial), while the 0 66 H plants were significantly lower than the control However at the second reading, (30 days after burial), the CER of the 0 66 H plants was significantly higher than control. After 40 days of burial, both the 0 33 H and 0 66 H plants were still significantly higher in their CER as compared to the control plants, but the 0 66 H plants were significantly lower than the 0 33 H plants (Fig 4 1)

In *Xanthium strumarium*, at the first reading, (16 days after burial), the CER of 0.33 H plants was significantly higher than the control plants but that of the 0 66 H plants was significantly lower than the control and 0 33 H plants At 37 days after burial, the CER of the 0.33 H and 0 66 H plants was significantly higher than control (Fig 4 1 and Plate 4 2b)

In case of *Cakile edentula* only two sets of readings were taken because the leaves of plants had been detrimentally affected by aphids After 16 days of burial, the CER of the 0.33 H plants was significantly higher than control plants, and that of the 0.66 H plants was significantly higher than 0 33 H plants Significantly higher CER

Figure 4.1 Mean (± SE) carbon dioxide exchange rate (μmol $m^{-2}s^{-1}$) for eight common dune species on different dates after burial in sand to 0, 33 and 66% of their height under greenhouse conditions N=6


A. psammophilum
C. biennis
S. helvola
P. virgatum
E. canadensis
C. hyssopifolium
X. strumarium
C. edentula
Control
33%
66%
CER (μmol m⁻² s⁻¹)
TIME (days after burial)

readings were recorded for buried plants on the second reading (26 days after burial) (Fig 4 1, Plate 4 2c and 4 3b)

(ii) Growth Chamber - Although the general trend in the results of the growth chamber experiments were similar to those of the greenhouse experiments, there was less variance in the data probably owing to precise control over factors such as temperature, light intensity and relative humidity

Experiment I Constant temperature (25°C) and varying light intensities (500, 1000, 1500 and 2000 µmol $m^{-2}s^{-1}$)

A significant increase was observed in the CER readings at all the different light intensities for all species in the 0.33 H and 0.66 H plants over control plants (Fig 4 2) It was also clear that all the species had a higher CER at higher light intensities (1500 and 2000 µmol $m^{-2}s^{-1}$) In *Agropyron psammophilum*, *Strophostyles helvola* and *Elymus canadensis* the 0.33 H and 0.66 H plants showed a large increase in CER than control The increase was rather linear up to the 1500 µmol $m^{-2}s^{-1}$ of light intensity, but there was a drastic increase in the CER between 1500 µmol $m^{-2}s^{-1}$ and 2000 µmol $m^{-2}s^{-1}$ light intensity in all three species. It was also evident from Fig. 4.2 that there was a marked difference in the CER of all species between the control and buried plants

Oenothera biennis showed a sudden drop at the 1500 µmol $m^{-2}s^{-1}$ light intensity level and again increased significantly at the 2000 µmol $m^{-2}s^{-1}$ light intensity level. In case of control, the increase in CER was very sharp between 500 and 1000 µmol $m^{-2}s^{-1}$ light intensity levels, whereas at the higher light intensity the increase in CER was not so high

Cirsium pitcheri and *Panicum virgatum* showed a similar trend in their responses to varying light intensities In both species the 0.33 H plants showed greater CER than the 0 66 H plants at the 2000 µmol $m^{-2}s^{-1}$ light intensity level.

Experiment II Constant light (1500 µmol $m^{-2}s^{-1}$) and varying temperatures.

In this experiment it was evident that the optimum temperature for the highest CER varied with species For example, in *Panicum virgatum* 35°C was the optimum temperature while in *Oenothera biennis*, *Agropyron psammophilum* and *Strophostyles helvola*, and *Elymus canadensis*, 25-30°C seemed to be the optimum temperature (Fig.

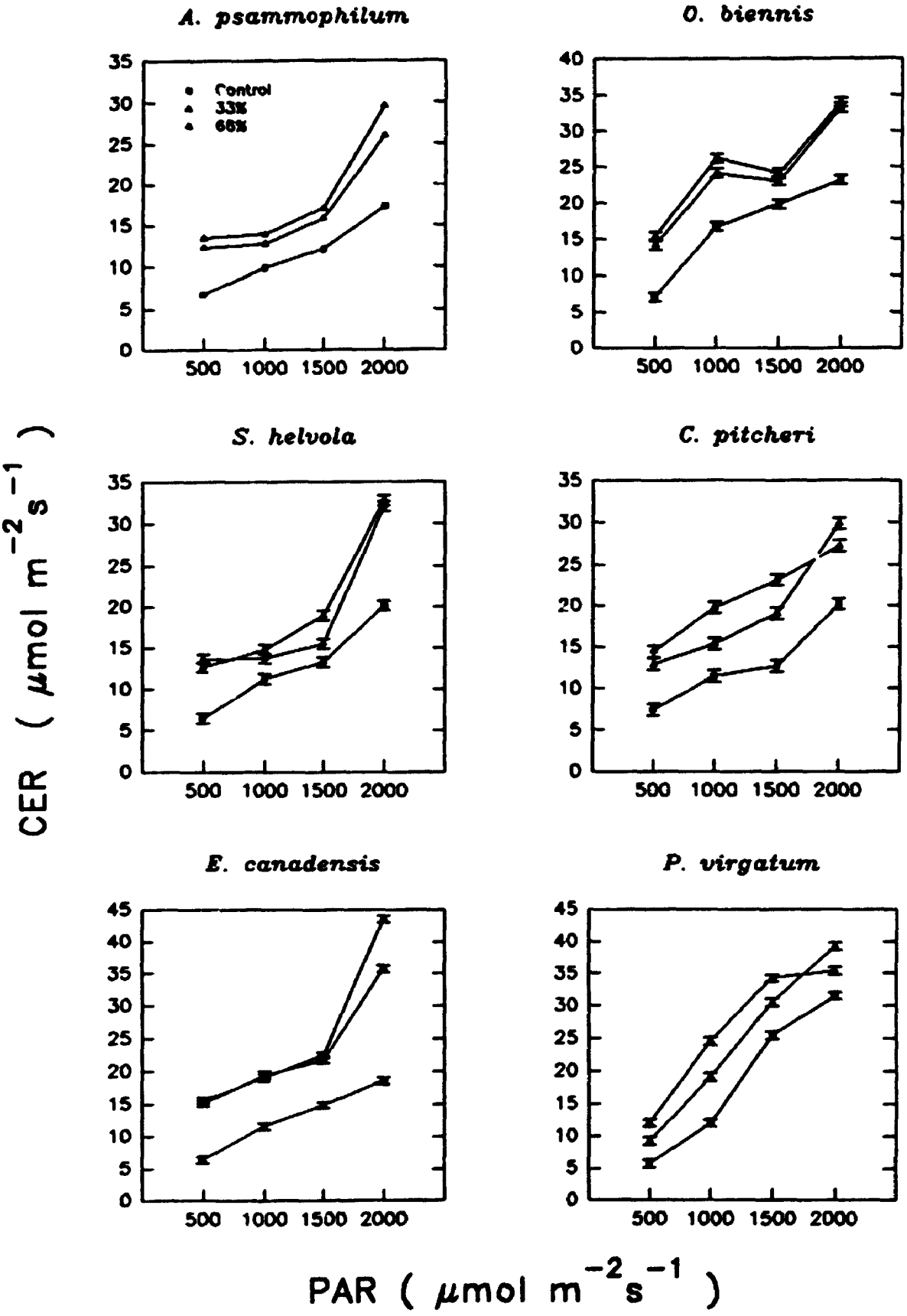
Plate 4.3 a *Panicum virgatum* from control (left), the 0.33 H plant in the centre and the 0.66 H plant (right) Treated plants flowered earlier than the control

b *Cakile edentula* control plant (left), the 0.33 H plant (centre) and the 0.66 H plant (right), following harvest An increase in biomass due to the burial treatments is evident in the picture


a


b

Figure 4.2 Mean (± SE) carbon dioxide exchange rate (μmol $m^{-2}s^{-1}$) of six common dune species in the growth chamber experiment at constant temperature (25°C) and varying light intensities of 500, 1000, 1500 and 2000 μmol $m^{-2}s^{-1}$ N=6


A. psammophilum
O. biennis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
Control
33%
66%
CER (μmol m⁻² s⁻¹)
PAR (μmol m⁻² s⁻¹)
500 1000 1500 2000

4.3) For *Cirsium pitcheri* the CER was not affected by changes in the temperature Here again, we saw that in all species the 0 33 H and 0 66 H plants showed much higher CER than the control (Fig 4 3) Except in the case of *Agropyron psammophilum* and *Panicum virgatum*, where the 0 33 H plants showed greater CER than the 0 66 H plants at the 35°C temperature, all other species had a greater CER in the 0.66 H plants than the 0 33 H plants at all temperature regimes In *Panicum virgatum* the increase in CER of buried plants were very sharp at high temperatures compared to control (Fig 4.3) *Strophostyles helvola*, *Elymus canadensis* and *Oenothera biennis* showed a definite decline in the CER at the 35°C temperature (Fig 4.3).

Experiment III. Constant temperatures and constant light intensities

There was a significant increase in the CER of buried plants as compared to control at constant temperature (25°C) and constant light intensity, (1500 µmol $m^{-2}s^{-1}$) There was also a significant increase in the CER in plants buried to 66% of their height over the 33% burial This was true for all three time periods in all species except *Agropyron psammophilum* and *E canadensis* at 20 days after burial (Fig 4 4)

There was a substantial increase in the CER of all species and in nearly all the burial treatments over time (Fig. 4 4). The only exception was *Oenothera biennis* in the 0 66 H treatment where CER measurements were linear.

The 0.66 H plants of *Agropyron psammophilum* and *Elymus canadensis* showed a decrease in the CER measurements at the 2nd reading (20 days after burial) compared to control plants. However in both species the 3rd reading (27 days after burial) showed a big increase in the CER measurements (Fig 4.4)

4.3.2 Effect of Sand Burial on the Photochemical Efficiency (F_v/F_m) of some common dune species under controlled conditions

(1) GREENHOUSE The photochemical efficiency varied among the eight species and between the control and buried plants *Agropyron psammophilum*, *Strophostyles helvola*, and *Panicum virgatum* showed higher photochemical efficiency (F_v/F_m) in the 0 33 H plants as compared to control on the 1st reading (25 days after

Figure 4.3 Mean (± SE) carbon dioxide exchange rate (μmol $m^{-2}s^{-1}$) of six common dune species in the growth chamber experiment under constant light intensity (1500 μmol $m^{-2}s^{-1}$) and varying temperatures (20, 25, 30 and 35°C) N=6

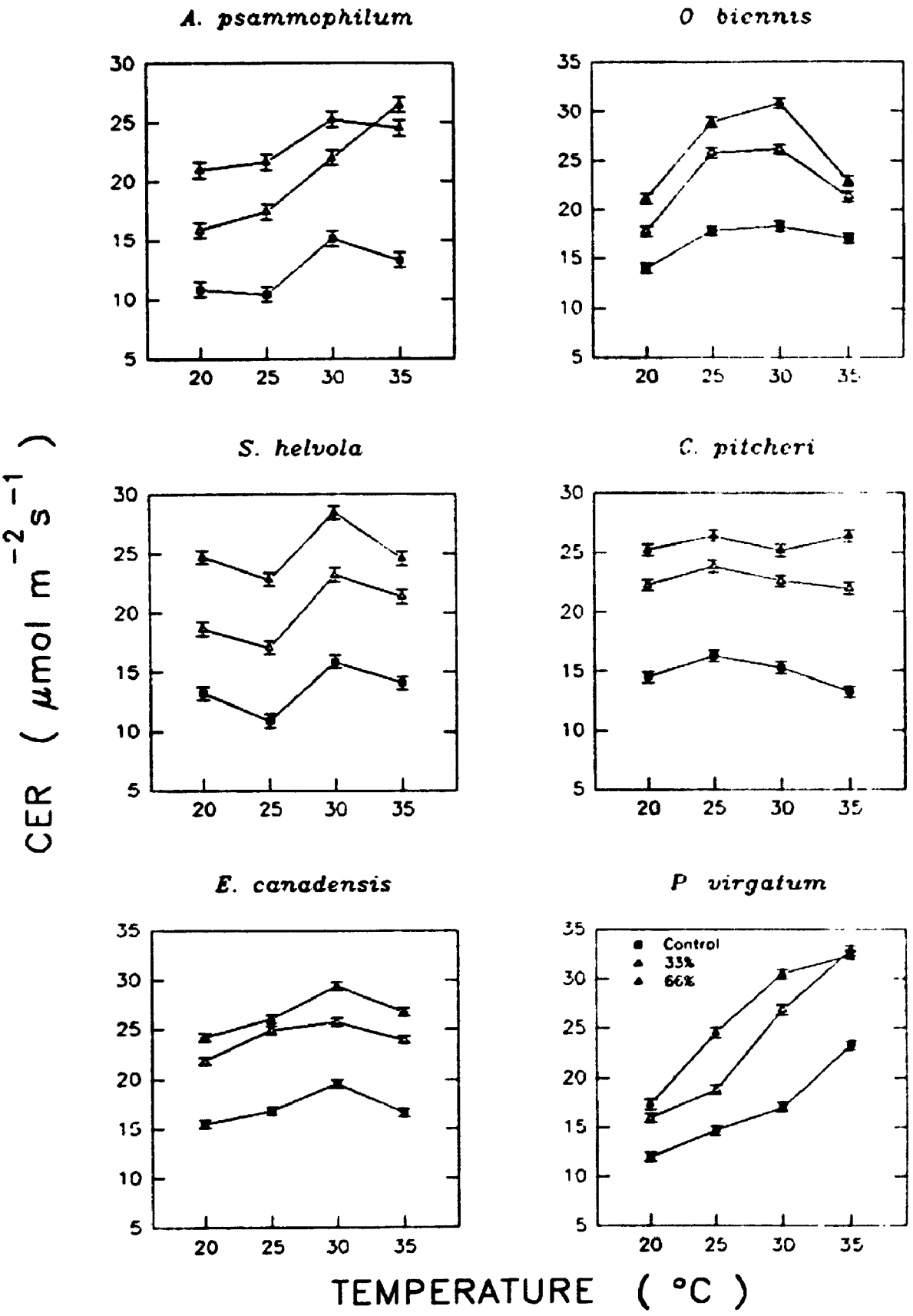
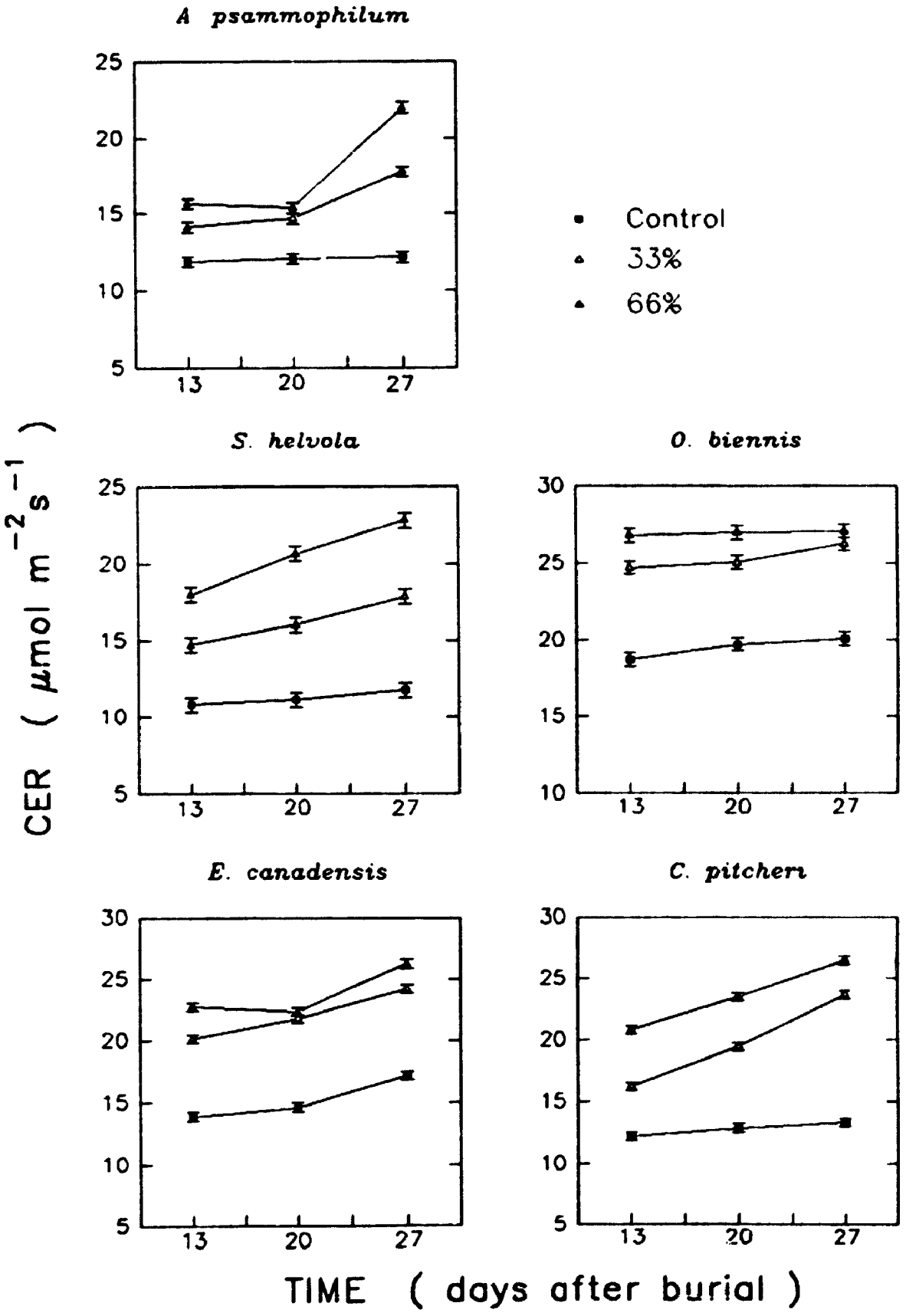

A. psammophilum
O biennis
S. helvola
C. pitcheri
E. canadensis
P virgatum
Control
33%
66%
CER (μmol m⁻² s⁻¹)
TEMPERATURE (°C)

Figure 4.4 Mean (± SE) carbon dioxide exchange rate (μmol $m^{-2}s^{-1}$) of five common dune species in the growth chamber experiment at constant light intensity (1500 μmol $m^{-2}s^{-1}$) and constant temperature (25°C) at 13, 20 and 27 days after the burial treatment. N=6


A psammophilum
S. helvola
O. biennis
E. canadensis
C. pitcheri
Control
33%
66%
CER (μmol m⁻² s⁻¹)
TIME (days after burial)
5
10
15
20
25
30
13
20
27

burial for *Agropyron psammophilum* and *Panicum virgatum* and 17 days for *Strophostyles helvola*) (Fig 4 5).

Agropyron psammophilum and *Strophostyles helvola* continued to show an increase in F_v/F_m values in the 0.33 H than control plants In both species there was a decrease in the F_v/F_m value in the 0.33 H plants compared to the control after 40 days of burial In *Strophostyles helvola* (45 days after burial) the 0.66 H plants had a higher F_v/F_m value than the 0 33 H plants In *Agropyron psammophilum* there was a significant decline after 59 days of burial in the F_v/F_m value of the 0.66 H plants compared to the 0.33 H and control plants.

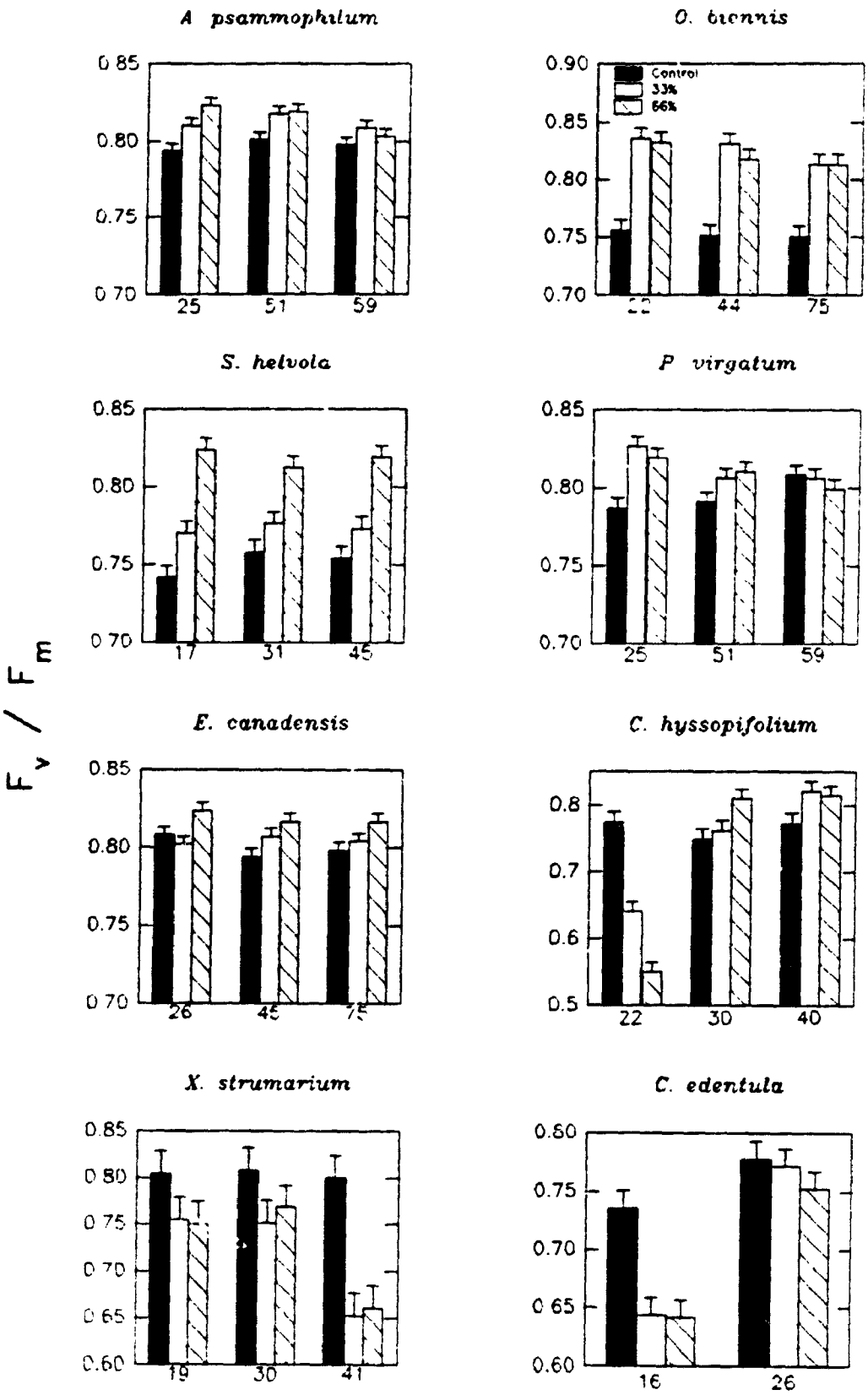
In case of *Panicum virgatum* there was a significantly higher F_v/F_m value for the 0 66 H plants compared to the control and 0 33 H plants 25 days after burial (Fig. 4 5) The later two readings were significantly lower for the buried plants as compared to control

Oenothera biennis did not show any difference between treatments on the 1st date of measurement (22 days after burial) but at the 2nd and 3rd readings (44 and 75 days after burial) there was a marked decline in the F_v/F_m for the 0.66 H plants compared to 0 33 H and 0.33 H plants compared to control. The F_v/F_m values showed a marked increase over time in all treatments (Fig. 4.5).

The buried plants of *Elymus canadensis*, *Corispermum hyssopifolium* and *Cakile edentula* showed a slight decline in the F_v/F_m values on the 1st date of measurement as compared to control. In *Elymus canadensis* and *Corispermum hyssopifolium* there was an increase in the F_v/F_m values of the 0 66 H plants as compared to the 0.33 H plants at the 1st reading. At the 2nd reading there was only a slight increase in the F_v/F_m value of the 0.33 H over the control plants of *Elymus canadensis* but in *Corispermum hyssopifolium* there was a significant increase in the F_v/F_m value of the 0 33 H and 0.66 H plants compared to the control plants. *Cakile edentula* continued to show a decline among the buried plants over the control at the 2nd reading, but the difference was not as drastic as compared to the 1st reading (Fig. 4 5)

In *Xanthium strumarium* the F_v/F_m readings for the buried plants and the control plants were about the same for the 1st reading (19 days after burial) but at the

Figure 4.5 Mean (± SE) photochemical efficiency (F_v/F_m) for eight common dune species under control and buried conditions in a greenhouse N=6


A. psammophilum
O. biennis
Control
33%
66%
S. helvola
P. virgatum
E. canadensis
C. hyssopifolium
X. strumarium
C. edentula
F_v / F_m
TIME (days after burial)

2nd and 3rd readings (30 and 41 days after burial), there was a sharp decline in the 0.66 H compared to the 0.33 H plants. At the second reading there was no marked increase between the control and the 0.33 H plants but in the 3rd reading there was a slight increase in the 0.33 H plants compared to the control plants but the difference was not significant (Fig. 4.5).

(ii) GROWTH CHAMBER

Experiment I. Constant temperature with varying light intensities

At constant temperature (25°C) and varying light intensities (500, 1000, 1500, and 2000 μmol $m^{-2}s^{-1}$), the buried plants of all species had a higher F_v/F_m value than the control plants (Fig. 4.6). The trends of change in F_v/F_m in all species except *Panicum virgatum* were similar. The photochemical efficiency increased up to 1500 μmol $m^{-2}s^{-1}$ light intensity level and then showed a decline at 2000 μmol $m^{-2}s^{-1}$. The decline was rather steep in *Agropyron psammophilum*, *Oenothera biennis* and *Elymus canadensis*. In *Panicum virgatum* photochemical efficiency continued to increase with increasing light intensity. The increase was high when the light intensity increased from 500 to 1000 μmol $m^{-2}s^{-1}$ followed by a slow increase (Fig. 4.6).

Experiment II. Constant light intensities with varying temperatures

At constant light intensity (1500 μmol $m^{-2}s^{-1}$) and varying temperature (20, 25, 30, and 35°C) again, as in the case of the previous experiment, all species showed higher F_v/F_m values for buried plants compared to the control (Fig. 4.7). All species except *Panicum virgatum* and *Cirsium pitcheri* recorded the highest F_v/F_m values at 25°C and then there was a decline at 35 and 35°C (Fig. 4.7).

Cirsium pitcheri showed a significantly higher F_v/F_m value at 20°C for the buried plants than the control. At 25°C the buried plants did not show any significant change from the 20°C temperature but the control plants had a much higher F_v/F_m value than at the 20°C level. At the 30°C level there was a significant difference in the F_v/F_m value between the 0.33 H plants and control plants, but not between the control and the 0.66 H plants. The F_v/F_m value increased at 35°C and again there was a significant difference in the F_v/F_m value between the control plants and the treated plants (Fig. 4.7). *Panicum virgatum* showed a significant difference in the F_v/F_m value in the treated plants as compared to control at 20 and 25°C. At 25°C the F_v/F_m value

Figure 4.6 Mean (± SE) photochemical efficiency (F_v/F_m) of six common dune species in the growth chamber experiment at constant temperature (25°C) and varying light intensities of 500, 1000, 1500 and 2000 µmol $m^{-2}s^{-1}$. N=4

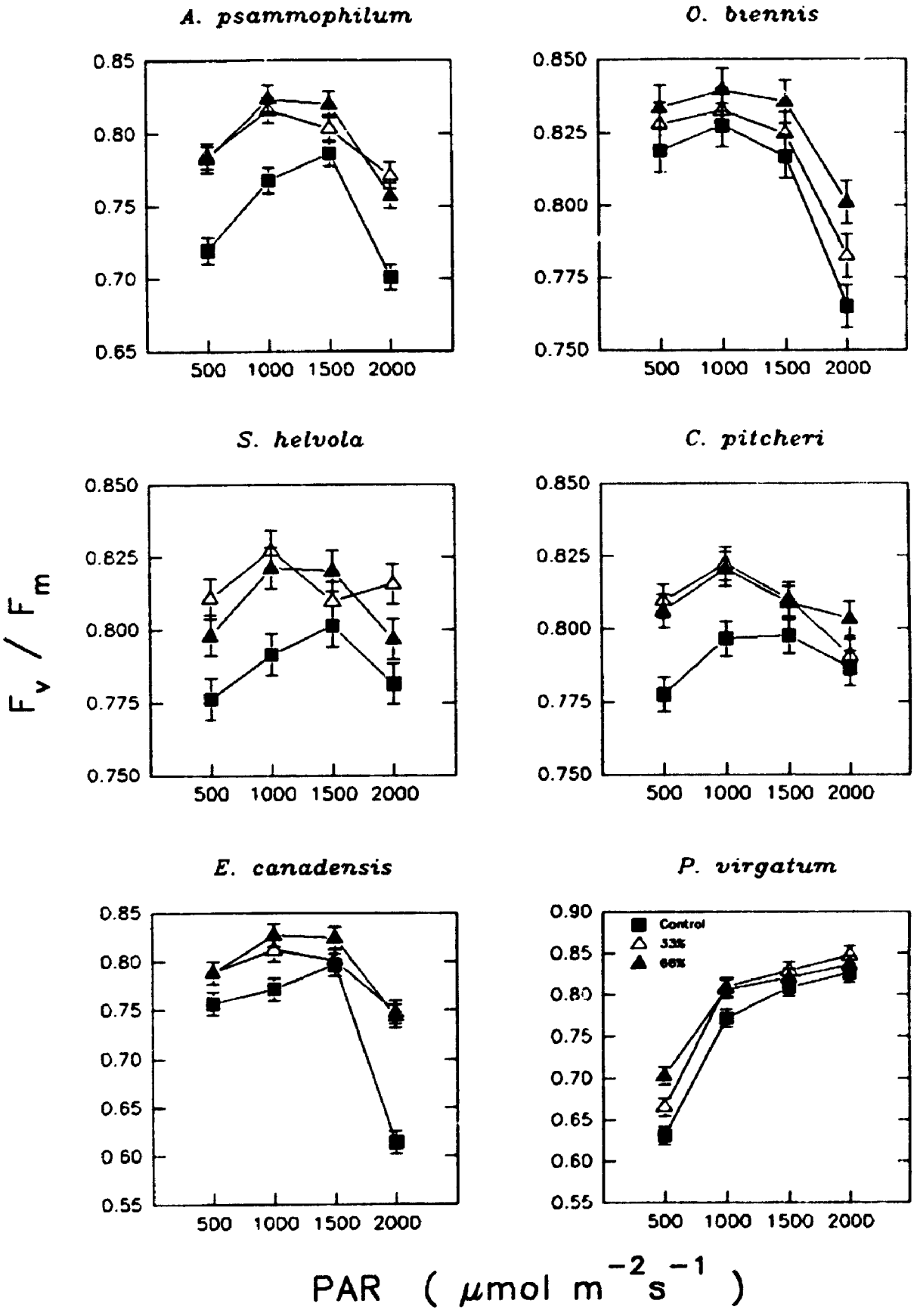
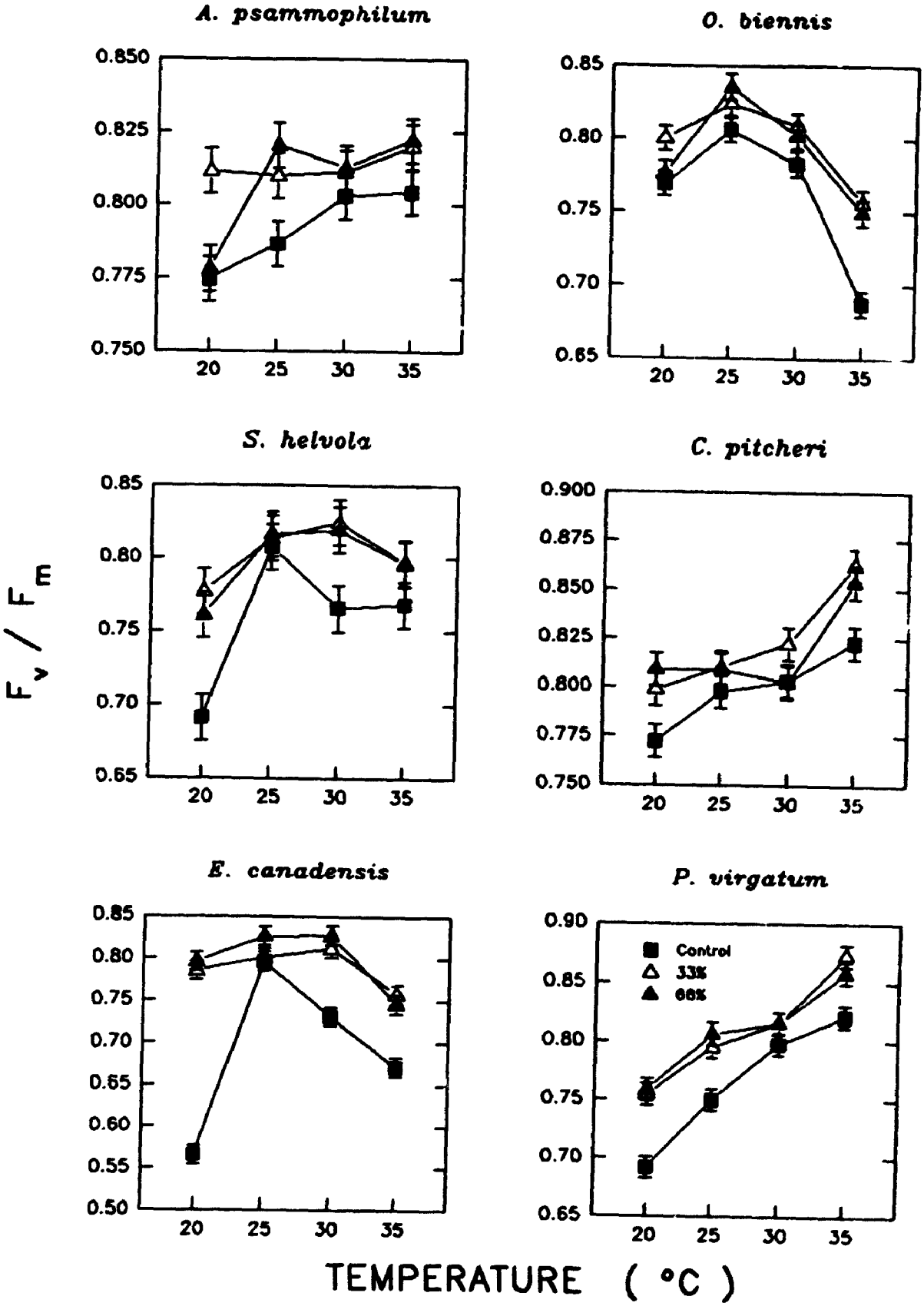

A. psammophilum
O. biennis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
Control
33%
66%
F_v / F_m
PAR (μmol $m^{-2} s^{-1}$)

Figure 4.7 Mean (± SE) photochemical efficiency (F_v/F_m) of six common dune species in a growth chamber maintained at constant light intensity (1500 µmol $m^{-2}s^{-1}$) and varying temperatures (20, 25, 30 and 35°C) N=4


A. psammophilum
O. biennis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
Control
33%
66%
F_v / F_E
TEMPERATURE (°C)

was higher than at 20°C, whereas at 30°C, although the F_v/F_m value were slightly higher, there was no significant difference between the control and the buried plants At 35°C there was a significant difference in the F_v/F_m value between the control and the buried plants and the values were also much higher than at lower temperatures (Fig 4 7)

Experiment III: Constant temperature and light intensities. At constant temperature and light intensity (13, 20 and 27 days after burial) there was a significant difference between the control and buried plants on the first date of measurement (13 days after burial) for all species, except for *Oenothera biennis*. On the subsequent date of measurement (20 days after burial) there was a significant difference between the buried and control plants only in *Elymus canadensis* (Fig 4.8) On the third date (27 days after burial) again the differences became evident between control and buried treatments in all species except *Strophostyles helvola* and *Cirsium pitcheri*. In *C. pitcheri* 66% burial treatment was significantly greater than control and 33% burial treatments (Fig 4 8)

4.3.3 Effect of Sand Burial on the Leaf Area, Biomass, and Root/Shoot Ratio of Several Common Dune Species Under Controlled Conditions

(i) GREENHOUSE- There was a significant increase in leaf area of buried plants over control plants of *Agropyron psammophilum*, *Oenothera biennis* and *Elymus canadensis* (Fig 4.9) In other species, *Cakile edentula*, *Panicum virgatum*, *Corispermum hyssopifolium*, *Strophostyles helvola* and *Xanthium strumarium*, the differences were not significant (Fig 4.9).

The dry biomass of buried plants, generally showed an increase over control but the differences were not always significant. In *Agropyron psammophilum* and *Xanthium strumarium* there was no significant difference between control and the 0 33 H plants, but the 0 33 H plants had were significantly higher biomass than the 0 66 H plants In *Strophostyles helvola* there was no significant difference in the biomass of the plants between the control and buried plants (Fig. 4.10). In *Oenothera*

Figure 4.8 Mean (± SE) photochemical efficiency (F_v/F_m) of five common dune species in a growth chamber maintained at constant light intensity (1500 μmol $m^{-2}s^{-1}$) and constant temperature (25°C) on different days (13, 20 and 27 days) after the burial treatment N=4

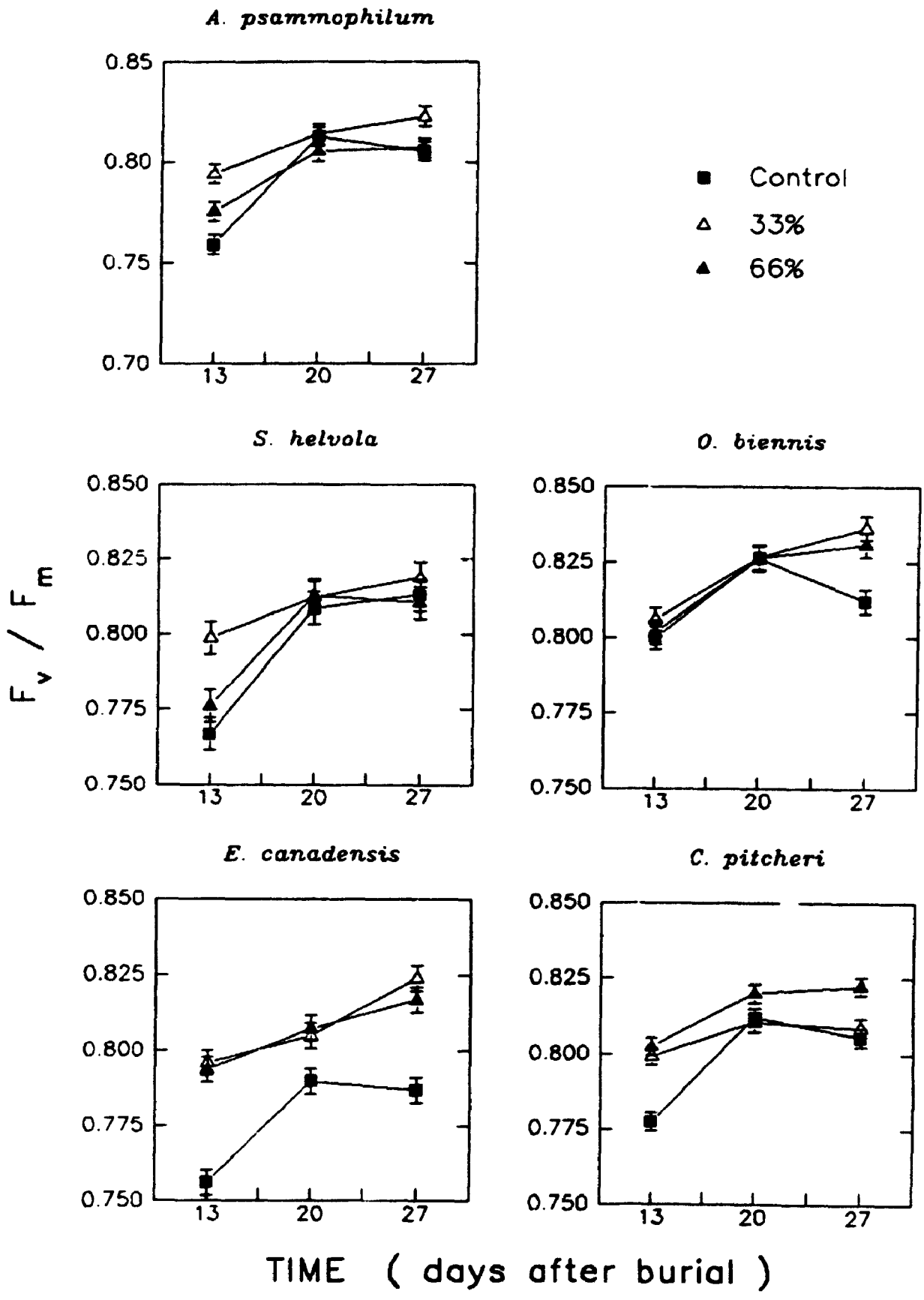

A. psammophilum
S. helvola
O. biennis
E. canadensis
C. pitcheri
Control
33%
66%
F_v / F_E
0.85
0.80
0.75
0.70
0.850
0.825
0.800
0.775
0.750
13
20
27
TIME (days after burial)

Figure 4.9 Mean (± SE) leaf area (cm^2) of eight dune species buried to depths of 0, 33 and 66% of their heights and harvested at the end of the greenhouse burial experiment N=6

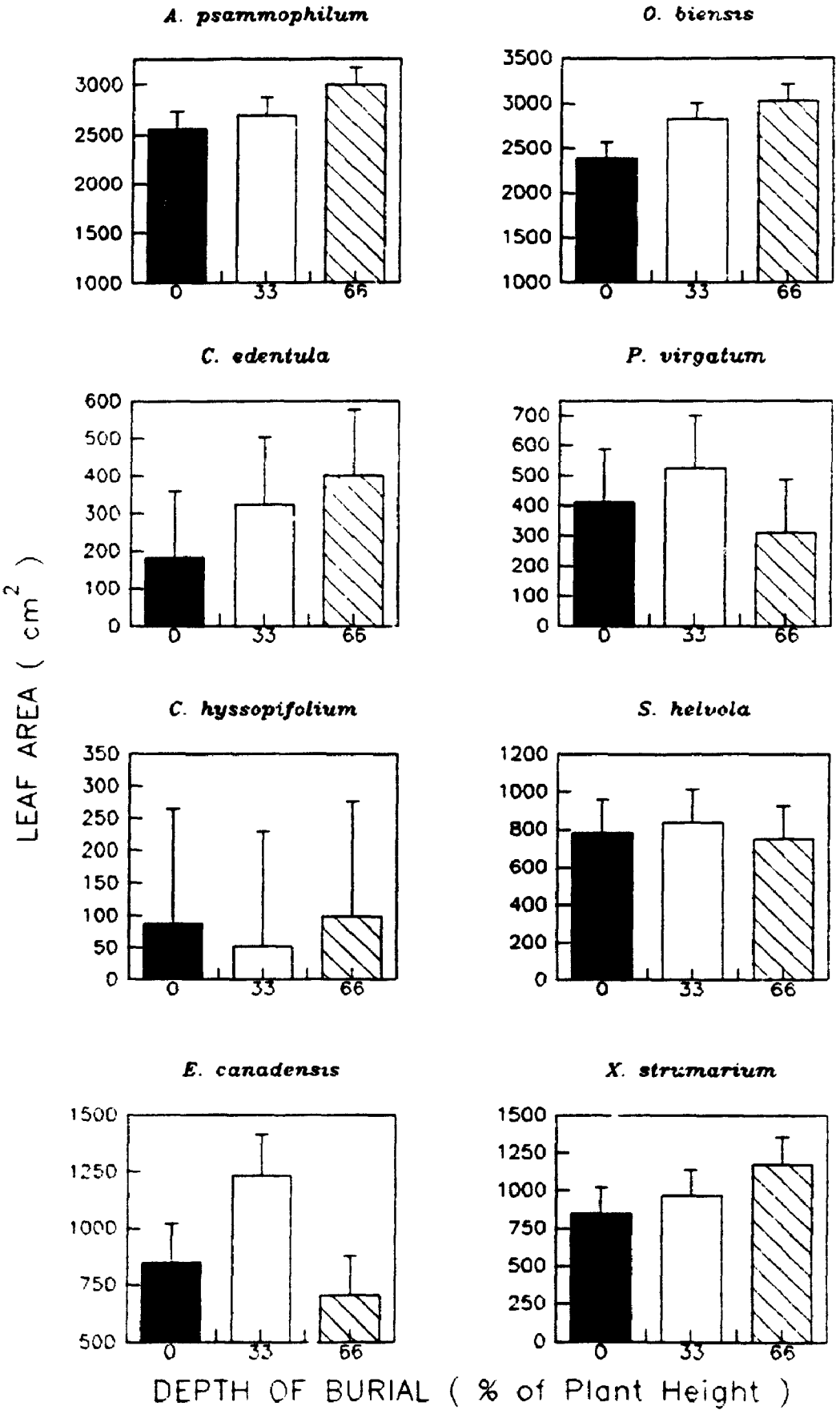
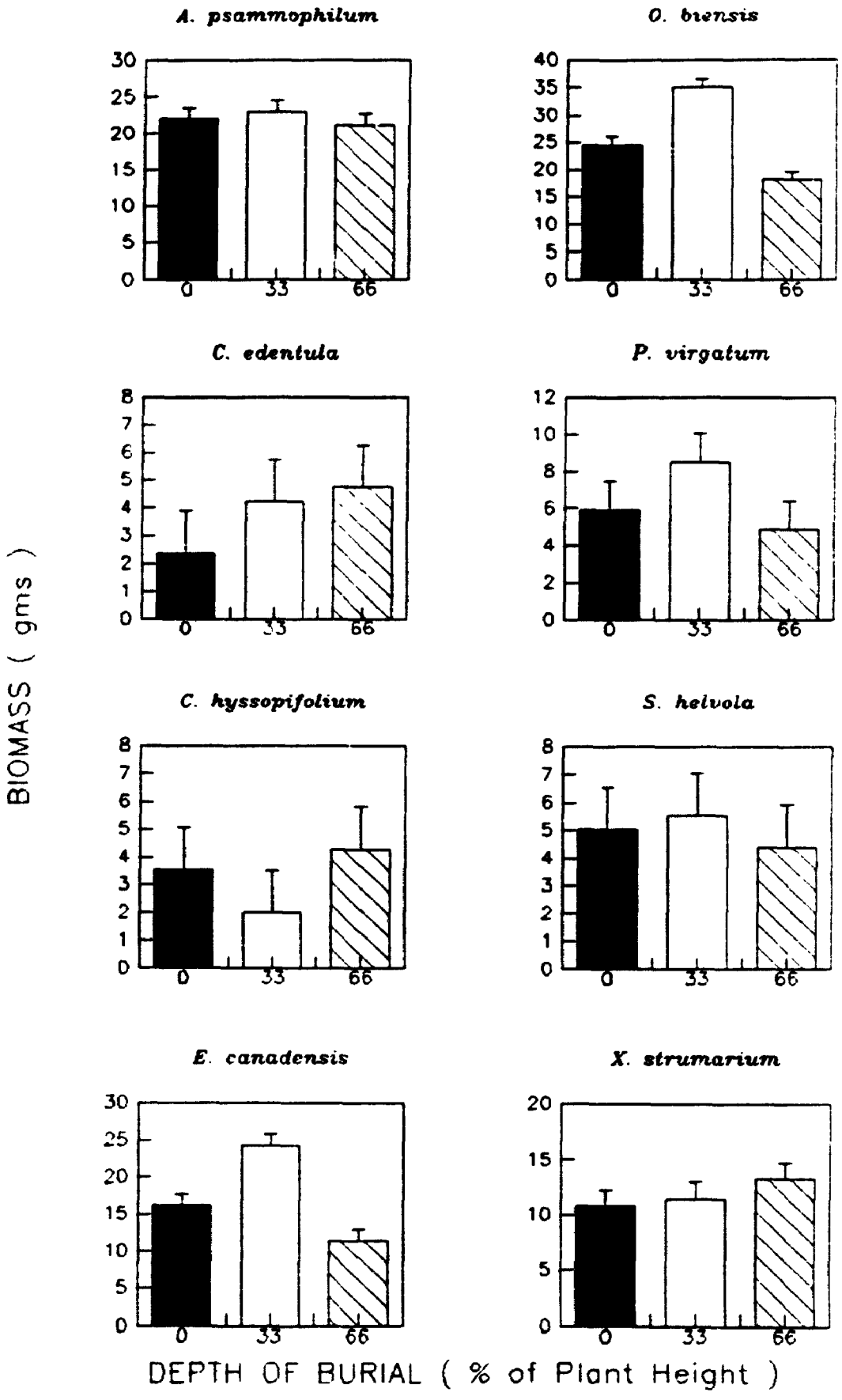

A. psammophilum
O. biensis
C. edentula
P. virgatum
C. hyssopifolium
S. helvola
E. canadensis
X. strumarium
LEAF AREA (cm 2)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

Figure 4.10 Mean (± SE) dry biomass of eight dune species buried to depths of 0, 33 and 66% of their height and harvested at the end of the greenhouse burial experiment. N=6


A. psammophilum
O. biensis
C. edentula
P. virgatum
C. hyssopifolium
S. helvola
E. canadensis
X. strumarium
BIOMASS (gms)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

biennis and *Elymus canadensis* there was a significant difference between the biomass of the control plants and the 0 33 H plants, but the 0 66 H plants had a significantly lower biomass than the 0.33 H plants.

The root/shoot ratio of plants in the greenhouse experiment showed a significant increase only in *Oenothera biennis* and *Cakile edentula* In *Corispermum hyssopifolium* there was a decrease in the root/shoot ratio In all other species there were no significant differences (Fig 4 11)

(ii) GROWTH CHAMBER Significant increase in the leaf area of the buried plants over control was obtained in all species except *Strophostyles helvola*, in which there was a significant decrease in the leaf area of the 0 66 H plants as compared to control and 0 33 H plants (Fig 4 12) In all the other species there was a significant increase in leaf area between control and 0 33 H plants as well as between 0 33 H and 0.66 H plants (Fig 4 12)

Generally speaking a significant increase in biomass between the control and buried plants in all species was observed There was a significant increase in the biomass of the 0.33 H plants the control and a significant increase in the biomass of 0.66 H plants over those of 0 33 H in all species with the exception of *Strophostyles helvola*, where 0 66 H plants had similar biomass to control but lower than 0 33 H plants (Fig 4 13).

The root/shoot ratio of buried plants was significantly higher than control in all the six species The root/shoot ratio of the 0 66 H plants was significantly higher than that of the 0 33 H plants, with the exception of *Panicum virgatum*, where the 0 66 H plants had a slightly lower root/shoot ratio than the 0 33 H plants but the difference was not significant (Fig 4 14)

Figure 4.11 Mean (± SE) root/shoot ratio of eight dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the greenhouse burial experiment N=6

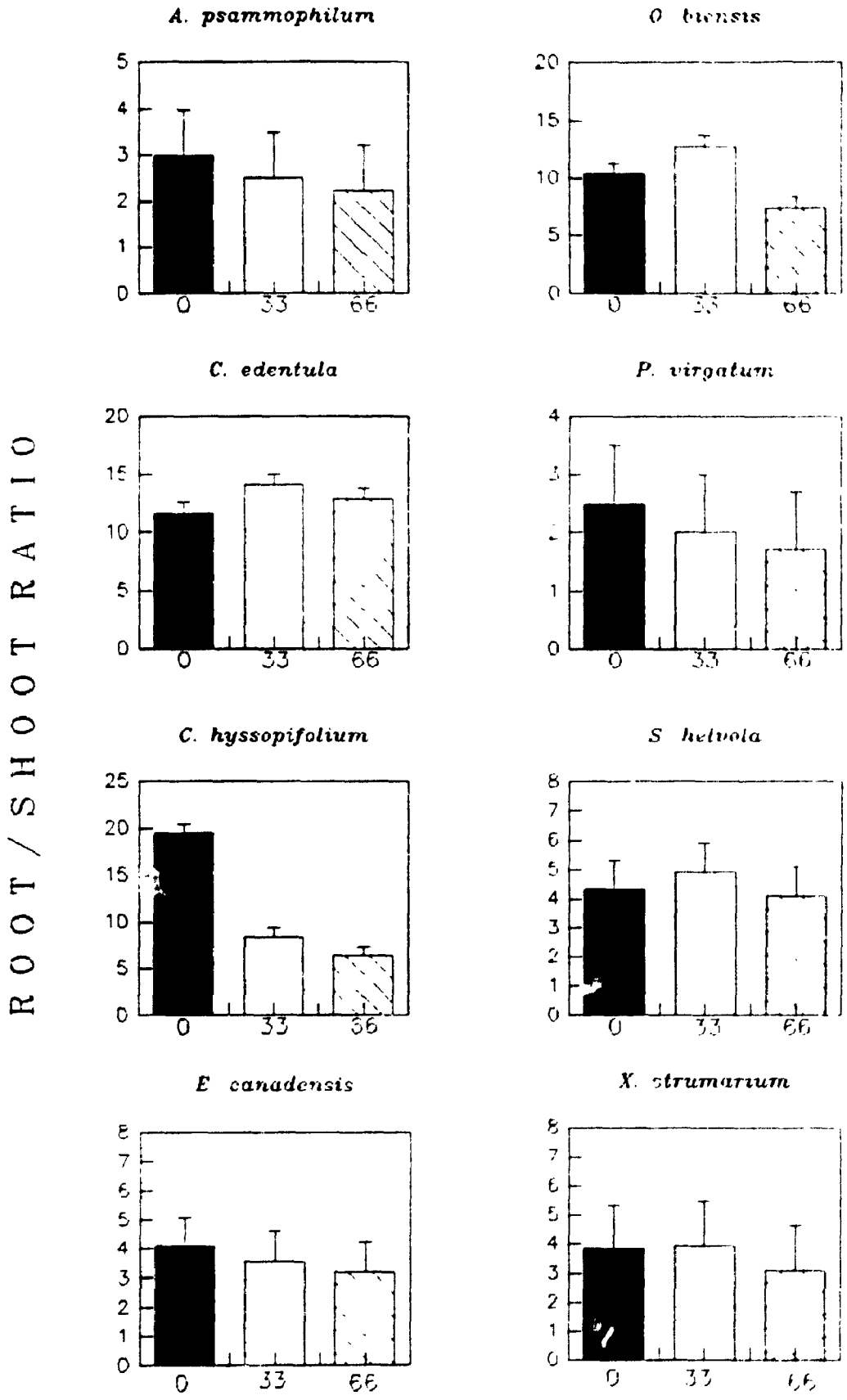

A. psammophilum
O. bursis
C. edentula
P. virgatum
C. hyssopifolium
S. helvola
E. canadensis
X. strumarium
ROOT / SHOOT RATIO
0
33
66
DEPTH OF BURIAL (% of Plant Height)

Figure 4.12 Mean (± SE) leaf area of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the growth chamber burial experiment. N=4

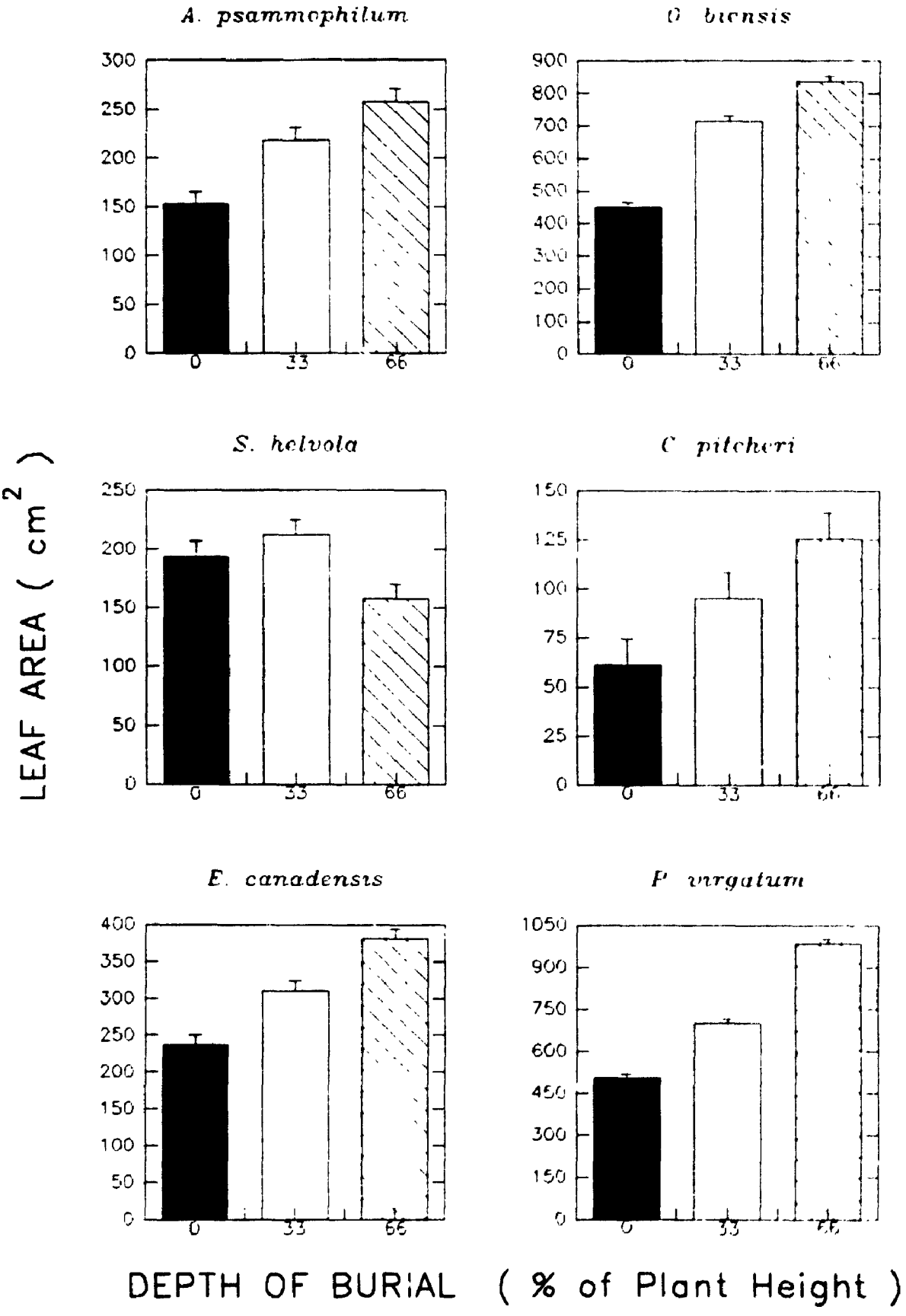

A. psammophilum
O. biensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
LEAF AREA (cm 2)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

Figure 4.13 Mean (± SE) dry biomass of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the growth chamber burial experiment N=4

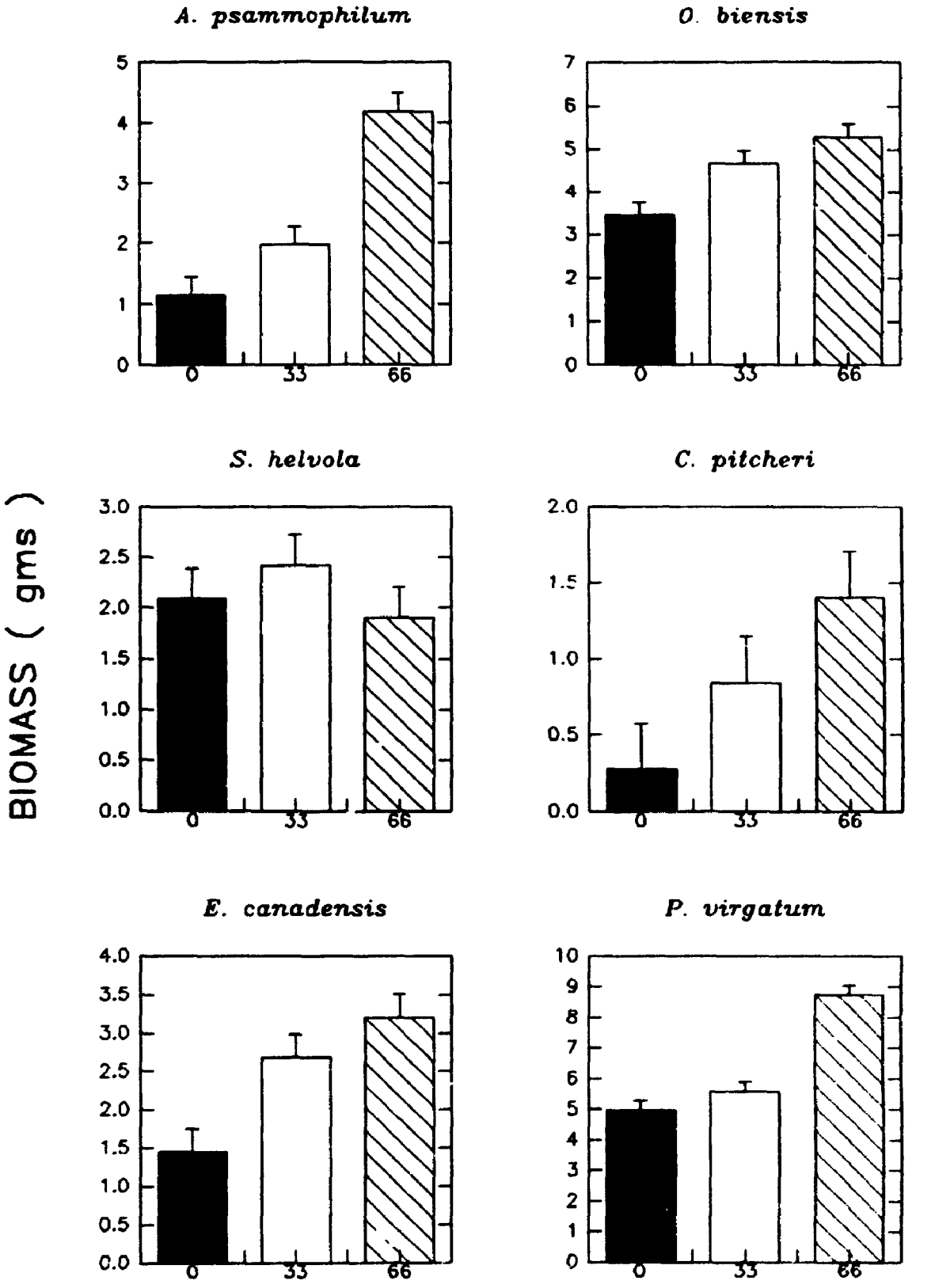
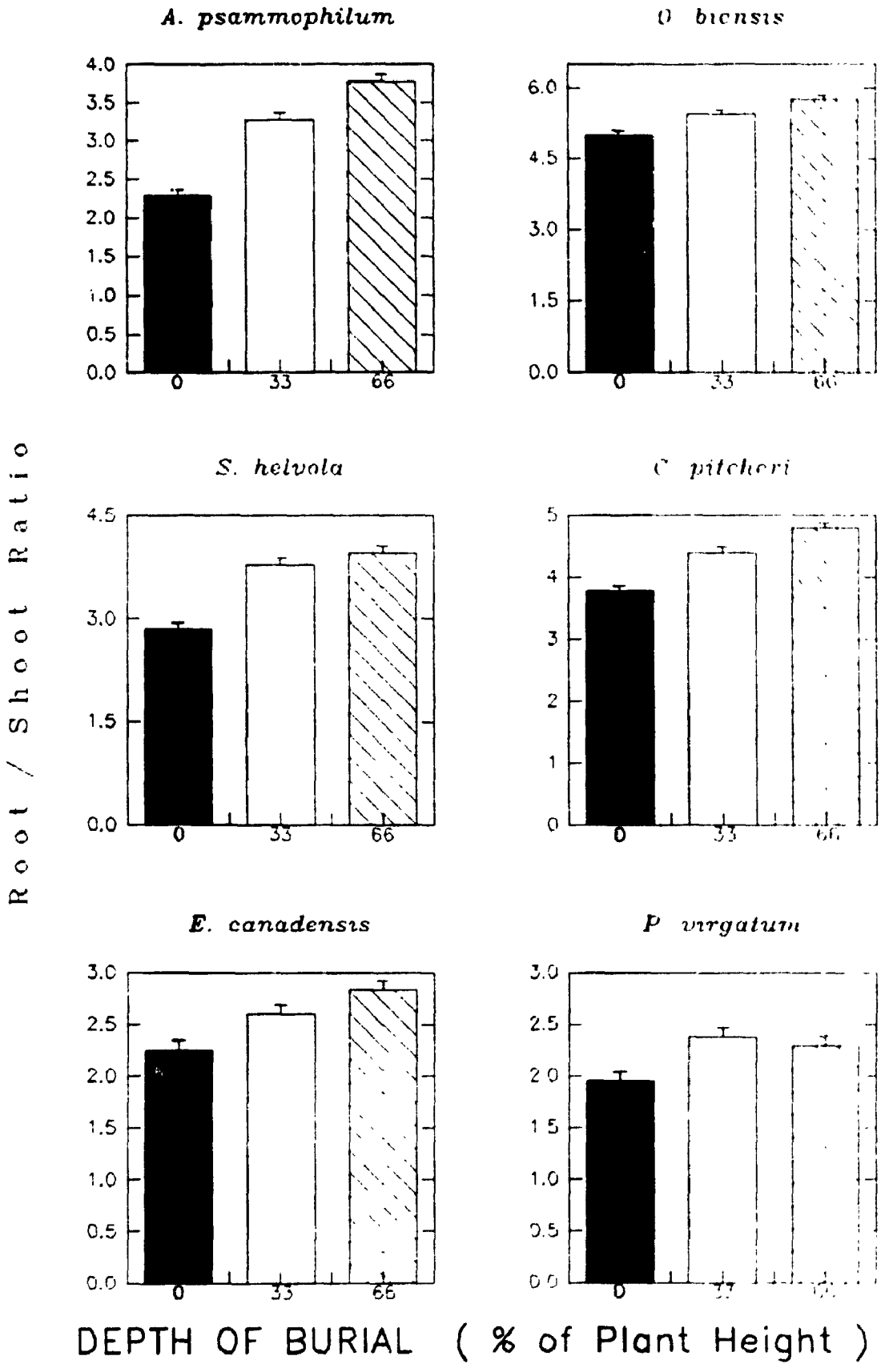

A. psammophilum
O. biensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
BIOMASS (gms)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

Figure 4.14 Mean (± SE) root/shoot ratio of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the growth chamber burial experiment N=4


A. psammophilum
O. biensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
Root / Shoot Ratio
DEPTH OF BURIAL (% of Plant Height)
0
33
66

4.3.4. Effect of Sand Burial on Total Chlorophyll Content, Chlorophyll a/b Ratio, and Leaf Thickness of Common Sand Dune Plants

The total chlorophyll content of buried plants was significantly higher than control plants of *Agropyron psammophilum, Elymus canadensis, Panicum virgatum* (Fig 4 15) In *Agropyron psammophilum* and *Panicum virgatum* there was no difference between the 0.33 H and the 0.66 H treatments whereas in *Elymus canadensis* the 0 66 H plants were significantly lower in their total chlorophyll content than 0 33 H plants In *Strophostyles helvola, Oenothera biennis and Cirsium pitcheri* there were no significant differences in the total chlorophyll content between buried and the control plants (Fig 4 15)

There were no significant differences between the 0.33 H and the 0.66 H treatments in *Oenothera biennis* and *Panicum virgatum* However, chlorophyll a/b ratio of *Cirsium pitcheri* leaves was significantly lower in the 0.66 H treatment as compared to the 0 33 H plants *Agropyron psammophilum, Strophostyles helvola* and *Elymus canadensis* plants did not show any significant differences between control and the buried plants (Fig 4 16)

All six species tested showed a significantly greater leaf thickness in buried plants in comparison to control In *Strophostyles helvola* and *Panicum virgatum* there were no significant difference in the chlorophyll a/b ratio between the two treatments (0 33 and 0 66 H) The thickness of leaves of plants in the two burial treatments were very similar in all species except *Agropyron psammophilum, Elymus canadensis, Oenothera biennis* and *Cirsium pitcheri* in which cases 0 66 H plants had greater leaf thickness than those of 0 33 H (Fig 4 17)

Figure 4.15 Mean (± SE) total chlorophyll content of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the greenhouse burial experiment N=4

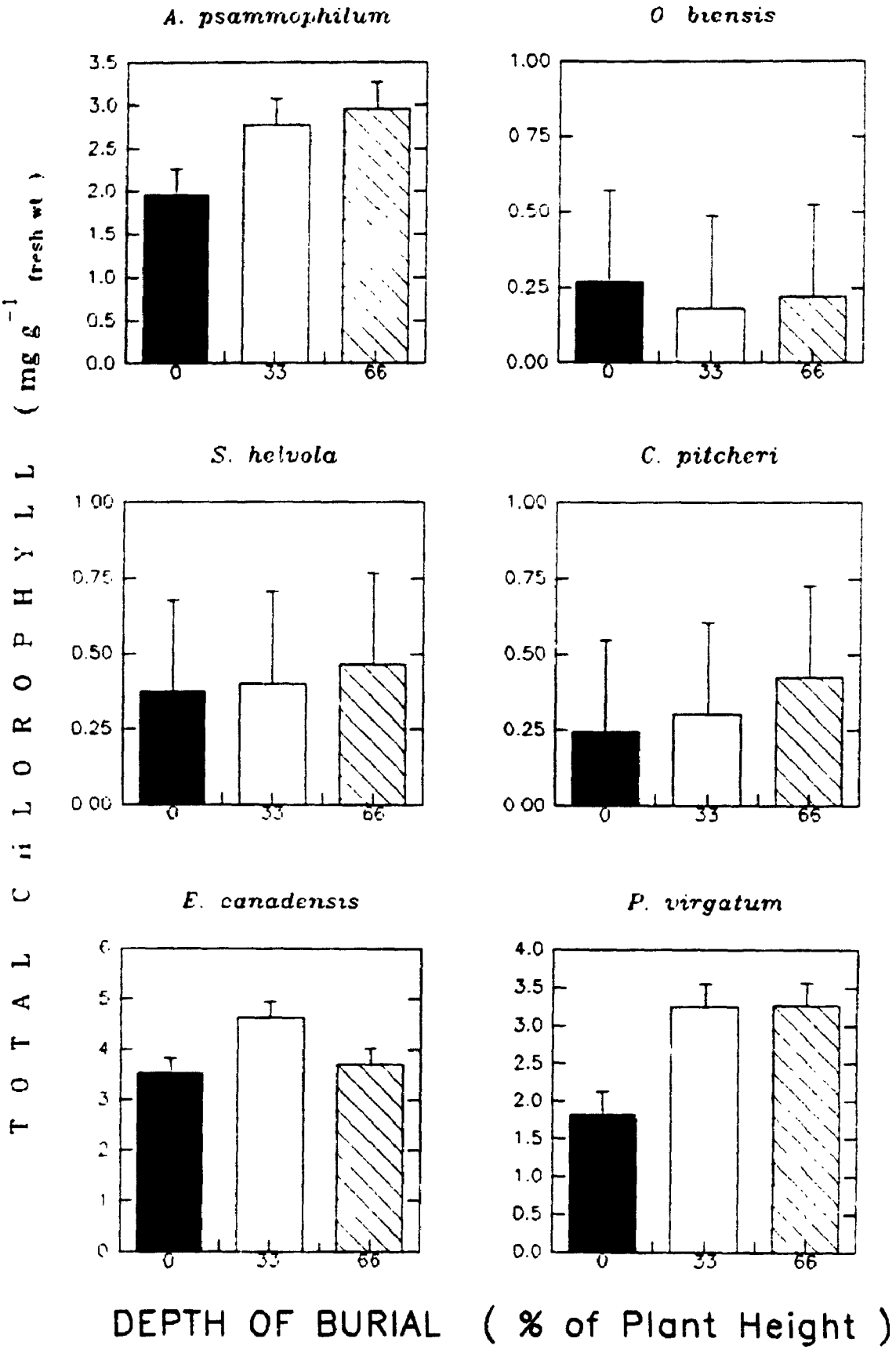

A. psammophilum
O brensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
TOTAL CHLOROPHYLL (mg g⁻¹ fresh wt)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

Figure 4.16 Mean (± SE) chlorophyll a/b ratio of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the greenhouse burial experiment N=4

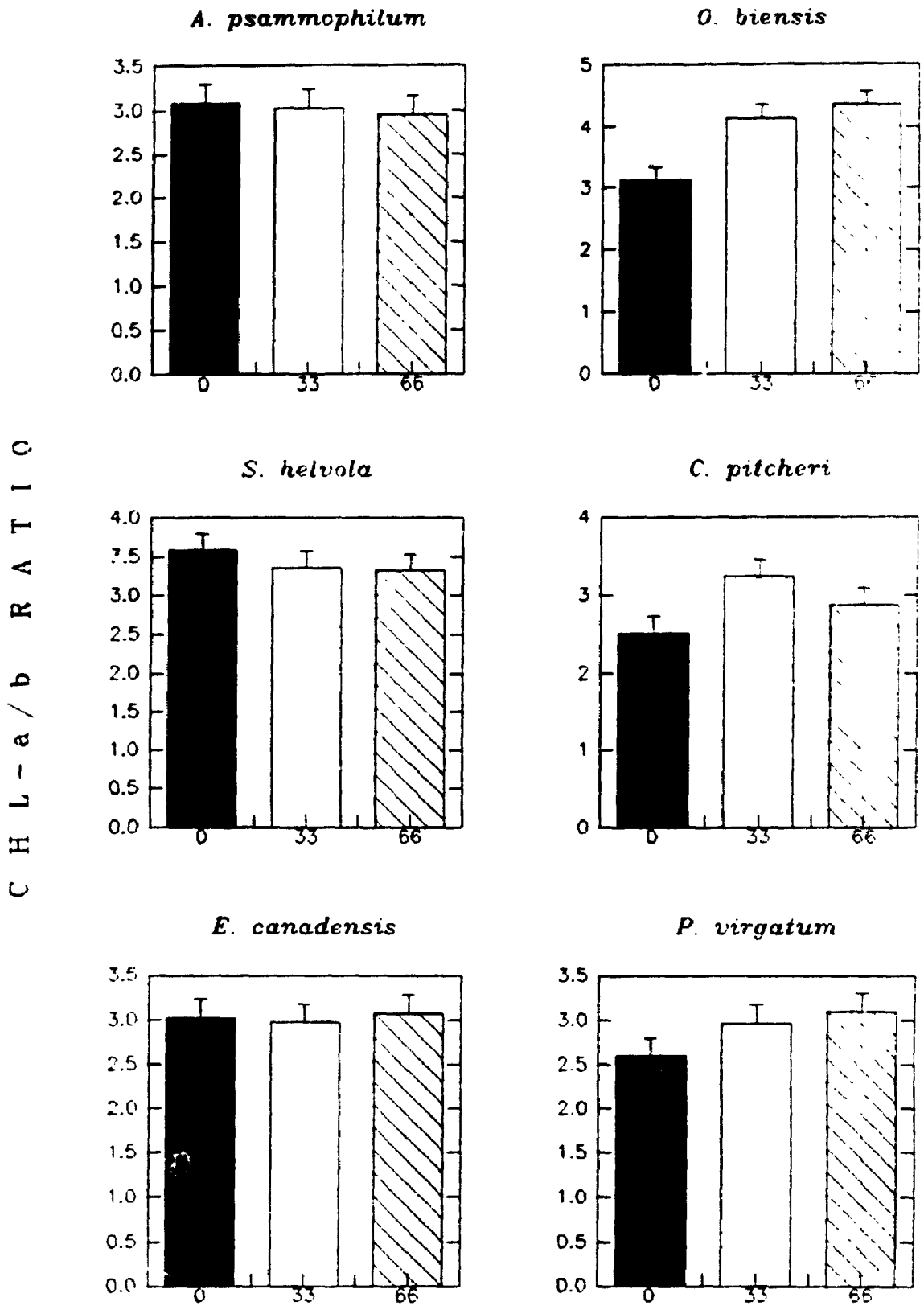
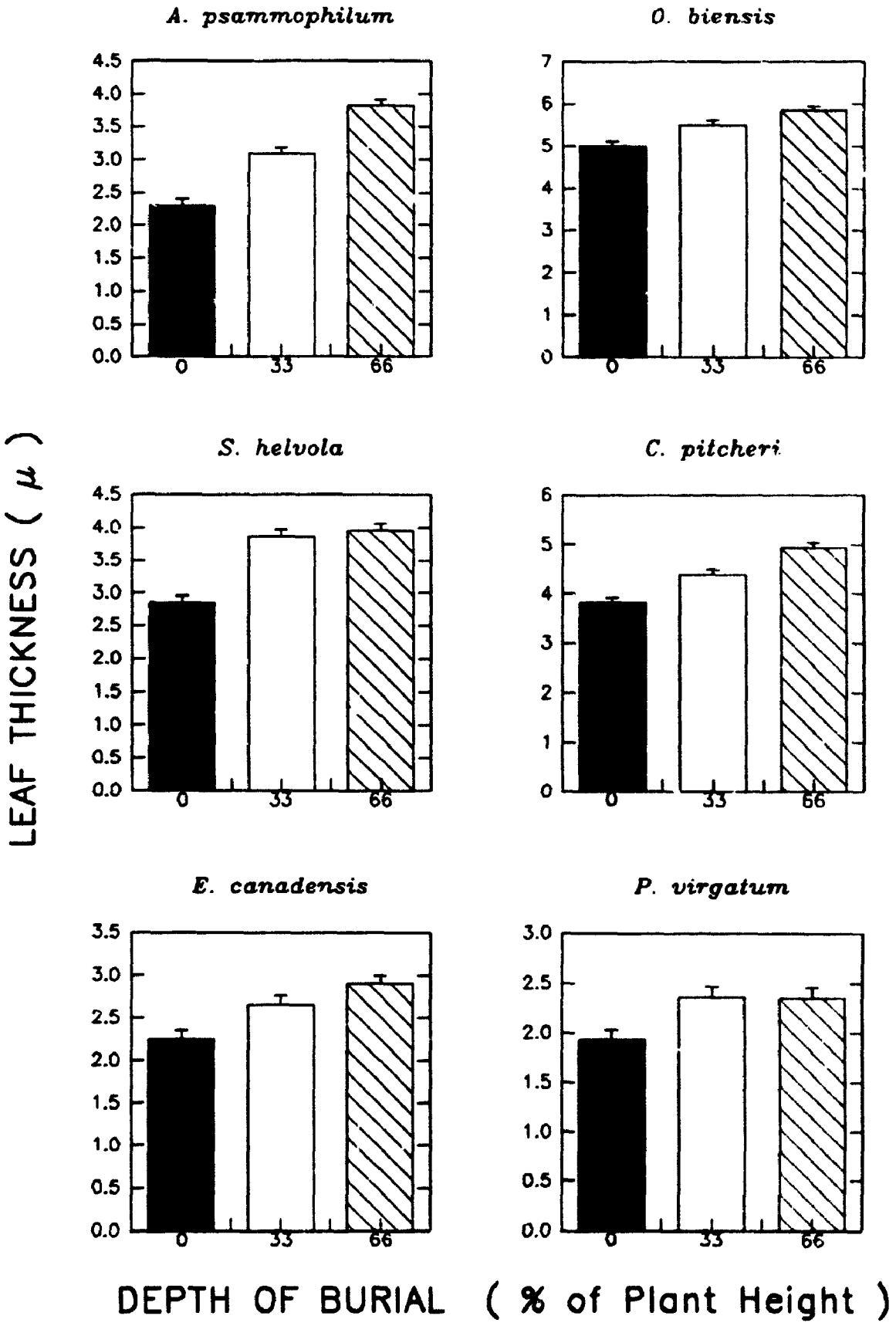

A. psammophilum
O. biensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
CHL-a/b RATIO
0
33
66
DEPTH OF BURIAL (% of Plant Height)

Figure 4.17 Mean (± SE) leaf thickness of six dune species buried to depths of 0, 33 and 66% of their height in sand and harvested at the end of the greenhouse burial experiment N=4


A. psammophilum
O. biensis
S. helvola
C. pitcheri
E. canadensis
P. virgatum
LEAF THICKNESS (μ)
DEPTH OF BURIAL (% of Plant Height)
0
33
66

4.4 Discussion

The experiments conducted under greenhouse and growth chamber conditions showed a definite increase in the CER (carbon dioxide exchange rate) of buried plants as compared to control This trend was seen in all the test species. This led to the acceptance of the hypothesis that partial sand burial enhances the CER of plants adapted to burial in the dunes There were, nevertheless, significant variations in responses to the various depths and durations of burial Light intensities and temperature regimes also made significant differences in CER In spite of the differences there was a definite trend towards higher CER in buried plants as compared to the control. The data gathered from this investigation clearly suggest that increased vigour of buried plants (Maun and Baye, 1989) can be partially explained by a rise in the level of carbon assimilation.

It has been suggested by several researchers that sand deposition alters temperature, moisture and light intensity of the microhabitat (Maun and Lapiere, 1984, Sykes and Wilson, 1990, Harris and Davy, 1988). Light intensity, moisture and temperature regimes are among the most important factors in photosynthesis and regulation of plant responses (Salisbury and Ross, 1992). Under natural conditions, buried plants were no doubt exposed to slightly higher light intensities after emergence because burial lowered the density of shoots and increased open areas around the plants, which not only reduced shading, but also increased the temperature of the sand surface Increased light intensity was beneficial to plants as shown by higher CER values of plants exposed to high light intensities. On sunny days temperatures, elevated above 25°C would be detrimental to most of the C_3 species tested in these studies However, in the C_4 species *Panicum virgatum*, the CER was almost directly proportional to an increase in temperature. C_4 plants are reported to be better adapted to higher temperatures (Salisbury and Ross, 1992)

Two aspects of leaf morphology, leaf area and leaf thickness were analyzed through the greenhouse and growth chamber experiments All the species investigated showed a significant increase in leaf area under growth chamber conditions. The

increase in leaf area may be one of the reasons why sand burial enhances the performance of dune species Similar results were also obtained for *Ammophila breviligulata* and *Calamovilfa longifolia* by Yuan *et al*, (1993) They pointed out that the optimum temperature for *Ammophila breviligulata* was elevated from 15-20°C in control plants to >20-25°C in buried plants Larger leaf area gives a greater leaf area index, enhances gross photosynthesis and carbon gain but would increase total evapotranspiration (Yuan *et al*, 1993) and respiration (pers comm N Huner) However, it must be pointed out that plants adapt to such environmental pressures by developing thicker cuticles that reduce transpiration and develop larger root systems that compensate for water loss (Yuan *et al*., 1993) Moreover, transpiration is a worthwhile investment on the part of the plant, since the greater leaf area increases the energy available through photosynthesis (Salisbury and Ross, 1985) Olson (1958a, b) and Yuan *et al*, (1993) have suggested that larger leaf area and leaf thickness could be due to lower water stress in buried plants Data from our studies showed that generally net photosynthetic rate decreased with higher stomatal resistance for all treatments, but at the same stomatal resistance buried plants had higher CO_2 uptake than control plants

Results of this experiment revealed that buried plants had higher photochemical efficiency than control in all species tested in the growth chamber and in most of the species in the greenhouse Irrespective of light intensity and temperature regimes the F_v/F_m was always higher in buried plants as compared to the controls. This indicated that the photosystem II of the photosynthetic apparatus in the buried plants were more efficient than control. This probably explains why there was a higher CO_2 uptake and greater rate of photosynthesis in buried plants Another conceivable reason for the increase in CER and F_v/F_m of buried plants was a compensatory response to the environmental stress caused by burial treatment (Yuan *et al*., 1993)

The increased rate of CER and F_v/F_m is further substantiated not only by leaf morphology but also by the increased biomass of buried plants Maun and Lapierre (1984) found that there was a significant increase in dry weight per shoot in *Ammophila breviligulata* with increased burial depth. The present work has also showed an increase in the biomass of all six species investigated in the growth

chamber and most species in the greenhouse This could be attributed to the increased photochemical efficiency and higher rate of photosynthesis

In conclusion, experiments under controlled conditions have reiterated the results of the field experiments Furt.. .more, it was evident that the rate of photosynthesis was related to the intensity of light and the response of the different species to light intensity though varied, had a similar general trend. Buried plants of all species tested had a higher CER and F_v/F_m It was also evident from this work that the F_v/F_m of the plants tested was low at low temperatures and constant light intensity. This could be due to photoinhibition which is prevalent at lower temperatures and high light intensities Leaf thickness of buried plants was greater in all six species in the growth chamber experiment as compared to control. The total chlorophyll content in *Agropyron psammophilum*, *Elymus canadensis* and *Panicum virgatum* leaves was higher than the controls In contrast, the total chlorophyll content of control and buried plants of *Cirsium pitcheri*, *oenothera biennis* and *Strophostyles helvola* did not show any differences

CHAPTER FIVE

IMPORTANCE OF VA-MYCORRHIZAE TO DUNE PLANTS

5.1 Introduction

Root-inhabiting microorganisms play a significant role in influencing the overall performance of plants. For example plant-parasitic nematodes and vesicular-arbuscular mycorrhizae (VAM) are known to co-exist in the roots of the same plant each having a characteristic but opposite effect on plant vigour (Allen, 1991). The obligate VAM fungi stimulate plant performance, while plant-parasitic nematodes usually suppress plant growth. VAM fungi are associated with a wide variety of plant species and have a much wider geographic distribution than any other type of mycorrhizal fungi Arbuscules produced by these fungi (develop from repeated dichotomous branching of intracellular hyphae), are considered to be major sites of nutrient exchange. Vesicles (spherical swellings at the tips of intercellular hyphae) most likely serve as storage organs (Hussey and Roncadori, 1982). VAM have a wide range of hosts and therefore are prevalent among cultivated crops as well as in natural plant communities (Gerdemann, 1975)

In spite of the harsh conditions of sand mobility, several species of plants are well adapted to sand dune habitat In fact, there is evidence that many species of foredune plants exhibit enhanced growth under buried conditions According to Eldred and Maun (1982), some plants have become so specialized that they require regular burial to maintain high vigour. Several explanations have emerged over the years to explain the phenomenon but little experimental evidence is available for any of the theories. Koske *et al.*, (1975) and Nicolson and Johnson (1979), from their work in the dunes of Australia, Scotland and Canada concluded that VA mycorrhizae may play an important role in the ecology of dune plants. VAM has been reported to provide nutrients especially phosphates to many plants (Allen, 1991).

We hypothesize that the enhanced performance of buried plants may be due to the activity of VA mycorrhizal fungi The questions asked was whether or not VAM are responsible for the enhanced performance of buried plants and if they are, how important is their presence? The answers to these questions will provide a clearer understanding of the role of VAM in sand dune systems. There has been no work done on the possible role of VAM in enhancing growth of buried plants In this research we have examined, (i) the percentage of root colonization by VA mycorrhizal fungi in twelve dune species in the field and (ii) the role of VAM in the enhancement of growth and vigour of two dune grasses, *Agropyron psammophilum* and *Panicum virgatum* under greenhouse conditions

5.2 Methods and Materials

5.2.1 Survey of VAM Colonization

In late September of 1992 an experiment was initiated to investigate the extent of mycorrhizal infection of plants in the sand dunes of Port Burwell Provincial Park (Plate 5 1) Twelve common dune species: *Agropyron psammophilum*, *Ammophila breviligulata*, *Cakile edentula*, *Corispermum hyssopifolium*, *Elymus canadensis*, *Equisetum arvense*, *Melilotus alba*, *Oenothera biennis*, *Panicum virgatum*, *Strophostyles helvola*, *Tusilago farfara*, and *Xanthium strumarium* were selected for this investigation. Twenty plants of each species were randomly chosen from the beach and dune complex near the northwestern end of the Port Burwell provincial Park along Lake Erie An area of 10 cm^2 around each plant was dug to a depth of 30 cm and approximately 50 g of small and fine roots (1 mm in diameter) were collected from each plant The root samples were placed in plastic bags, labelled and then taken to the laboratory where they were thoroughly washed in deionized water and fixed overnight (12-16 hours) in formyl acetic alcohol (FAA). The FAA solution was prepared by mixing with 1050 ml water, 1500 ml 95% ethanol, 150 ml acetic acid (glacial), and 300 ml formalin (37-40%).

The roots were cut into 1 cm long pieces and dispersed in a 2 liter beaker

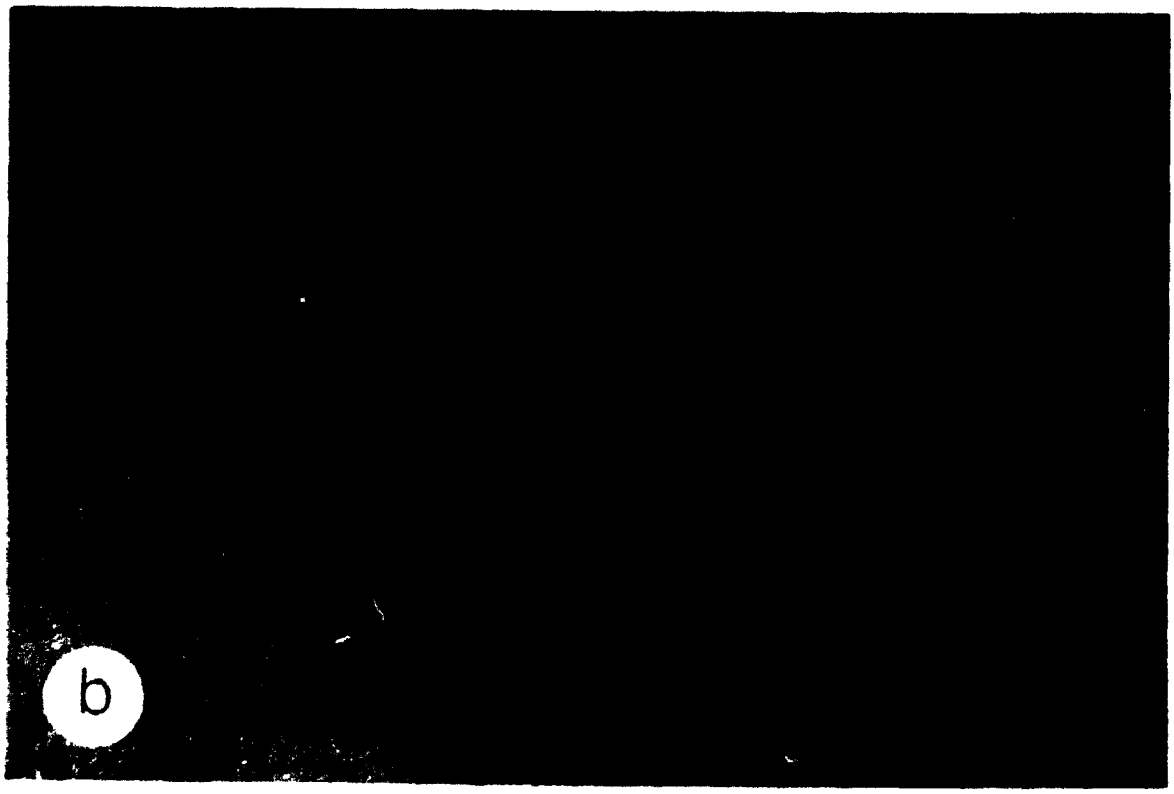
Plate 5.1 Sites at Port Burwell Provincial Park where twelve common dune species were sampled to determine VAM colonization on their root system

a. The diversity of dune species in the foredune plant community

b The large grass tussock in the foreground is *Panicum virgatum*

Photograph taken in summer, 1993


a


b

containing water. The water was stirred vigorously and a sub-sample collected using a 100 ml beaker This step was repeated until about 10 g of roots were obtained for making 3-4 slides. Root sub-samples were then immersed in 10% KOH (potassium hydroxide) and heated at 121°C for 7-12 minutes (depending on the texture of roots) The roots were then rinsed in deionized water and submerged in Chlorazol Black-E stain for 45 minutes to 3 hours in an oven at 50-60°C (Brundrett *et al.*, 1984).

The stained roots were then rinsed thoroughly in deionized water until all the extra stain was washed away They were then placed in glycerine, mounted on slides in parallel rows and covered with a 22 x 40 mm cover glass The slides were then examined with a compound microscope for assessment of VAM colonization using the magnified intersections method (McGonigle *et al.*, 1990)

5.2.2 Greenhouse Burial Experiment

An experiment was conducted to determine the effect of mycorrhizal fungi on the net CO_2 uptake, photosynthetic efficiency, and other morphological responses of buried and unburied sand dune plants. A preliminary burial experiment was conducted by planting seedlings of *Agropyron psammophilum*, *Elymus canadensis*, and *Panicum virgatum* in sterilized (pasteurized at 80°C for about 3 hr) and non-sterilized sands collected from the sand dunes The plants grown in the sterilized sand were more vigorous than those in the non-sterilized sand, probably due to the release of nutrients during the sterilization process. In consultation with Dr. Larry Peterson and Dr. Terry McGonigle of the University of Guelph we repeated the preliminary experiment using procedures established by Thompson (1990) Sand from the dunes was collected and sent to the nuclear reactor centre at McMaster University, Hamilton, Ontario, to be gamma radiated at 1 Mega rad. This level of radiation kills the VAM fungi in the sand (Thompson, 1990) Surface sterilized corn was germinated in an incubator (30°C, 14 hr light and 10 hr dark) and the seedlings were planted in sand collected directly from below *Panicum virgatum* and *Agropyron psammophilum* growing naturally in the sand dunes of Port Burwell Provincial Park along Lake Erie. A sample of roots from corn plants was harvested three weeks after it was planted and assessed for VAM colonization This procedure confirmed the presence of VAM in the roots of corn

plants All corn plants were then harvested and their roots were washed and cut into approximately 1 cm fragments. These fragments were used for the inoculation of plants in the following burial experiments.

Surface sterilized seeds of two common dune species, *Panicum virgatum* and *Agropyron psammophilum*, were germinated in an incubator and then planted in 8 cm long and 5 cm diameter PVC tubes filled with sterilized or unsterilized sand. The tubes were fitted with a 3.8 to 5 cm coupling on one side and a teflon net on the base (Plate 5.2) Before planting the seedlings in the PVC tubes, corn root fragments were placed below the seedlings to serve as inoculum for VAM fungi. Corn roots planted in sand from below *Agropyron* plants were placed in tubes where *Agropyron psammophilum* seedlings were planted and similarly corn roots planted in sand from beneath *Panicum* plants were placed below the seedlings of *Panicum virgatum*. The sand containing these inocula were referred to as VAM-containing sand. The seedlings were then placed on benches in a greenhouse maintained at 24°C day (14 hr) and 20°C night (10 hr) and a light intensity of approximately 350 µE.

The plants were given the following six treatments. Seedlings were grown in: i. (+) VAM-containing sand (VCS); ii. (-) VAM-free sand (VFS); iii. (++) VAM-containing sand and then buried with VAM-containing sand(VCS/VCS); iv. (+-) VAM-containing sand, buried with VAM-free sand (VCS/VFS); v. (--) VAM-free sand, buried with VAM-free sand (VFS/VFS) and vi (-+) VAM-free sand, buried with VAM-containing sand (VFS/VCS). First two treatments served as control. To prevent the movement of VAM fungi between the VCS and the VFS layers, a 0.45 µm pore size Millipore filter of 4.7 cm diameter was placed at the rim of the coupling in treatment (iv) and (vi)(VCS/VFS and VFS/VCS). The 0 45 µm pores prevented the penetration of VAM hyphae (Li *et al.*, 1991). After the fitting of filters the plants were buried to 50% of their height by using a 3.8 cm diameter PVC tube placed on the coupling (plate 5 2) Eight replicates were used for each treatment in the experiment

5.2.2.1 Measurement of Carbon dioxide Exchange Rate

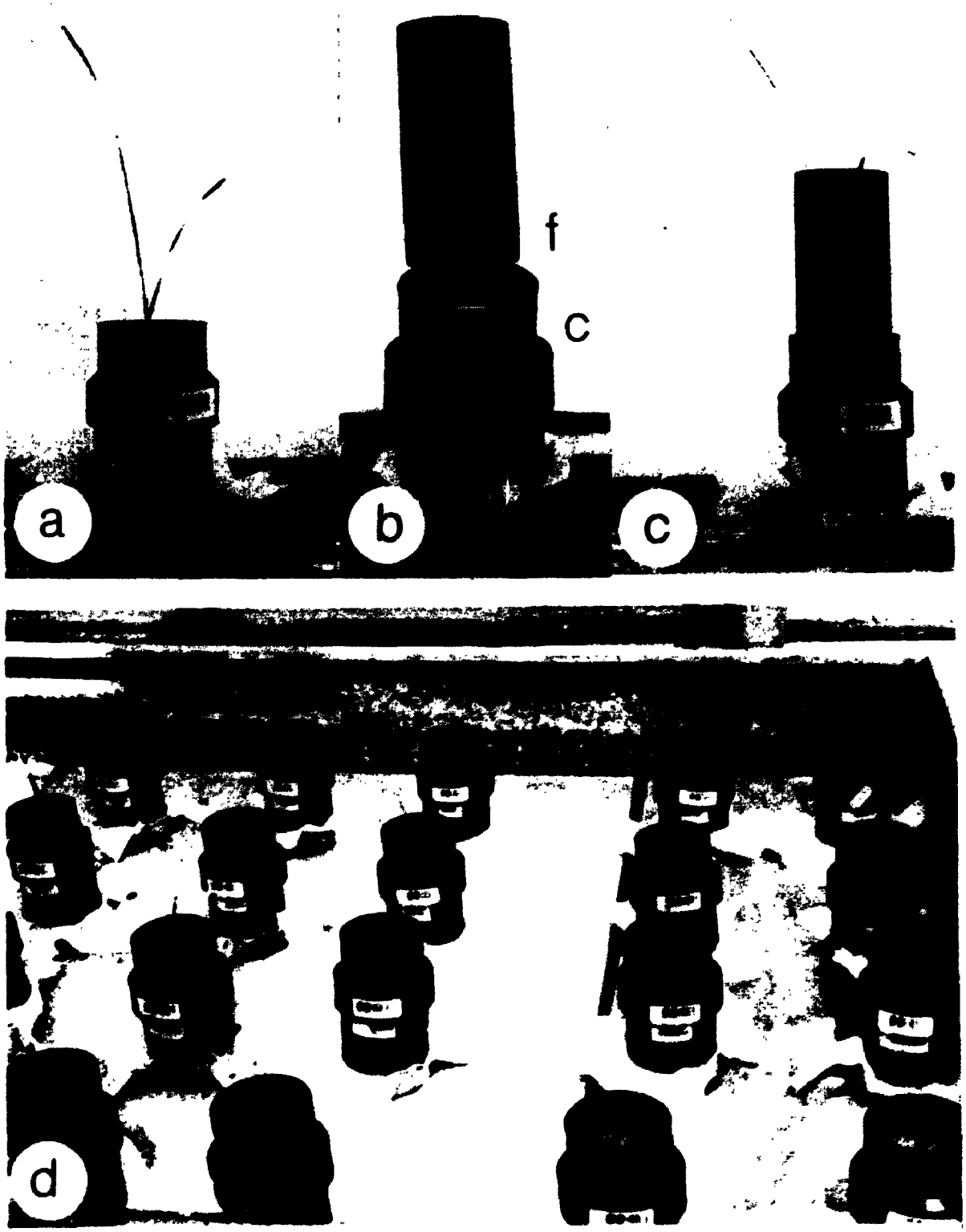
Carbon dioxide exchange rates (CER) were measured using the LI-COR

Plate 5.2 PVC tubes used in the burial experiment to determine the effect of VAM on buried plants.

a. An unburied plant of *Agropyron psammophilum* growing in a PVC tube;

b. Assembly of PVC tubes for the burial treatments with labels that indicate the following: f= 0.45μ millipore filter, c= coupling (1.5-2");

c. *Agropyron psammophilum* plant buried to 50% of its height, d Setup of burial experiment in which *Agropyron psammophilum* and *Panicum virgatum* plants were randomly placed on a greenhouse bench.


f
c
a
b
c
d

Portable Photosynthesis Gas Analyzer LI-6200 The CER measurements were an indication of the photosynthetic capacity of plants The first set of measurements were taken 14 days after the imposition of burial treatments on December 8 and 9, 1993 To minimize the variations in light intensity measurements were taken between 10 00 AM and 2 00 PM on sunny days Florescence lights were also used to increase the light intensity. Three readings were taken on each plant at each date of measurement The second and third sets of measurements were taken 20 and 26 days after the imposition of burial treatments, respectively In all instances the 2nd fully expanded leaf was used for the measurement

5.2.2.2 Photochemical Efficiency

Photochemical efficiency of PS II (F_v/F_m) was measured using a plant stress Meter (PSM) at 400 µE light level for 2 sec on the 2nd, 3rd and 4th fully expanded leaves on the same days as the CER measurements. On each day after the completion of the CER measurements the plants were dark adapted by placing in a dark room for about one hour The measurement was done by placing the leaf against the fiber optic end of the PSM The F_v/F_m measurement was an in vivo Photosystem II efficiency test, which indicates the photochemical efficiency of the plant

5.2.2.3 Plant height, leaf area and total plant biomass

On December 22, 1993, the greenhouse mycorrhizal burial experiment was terminated. First, the height of each plant was measured from the original soil surface to the tip of the 2nd fully expanded leaf. Then the leaves of each plant of *Agropyron psammophilum* and *Panicum virgatum* were carefully removed from the plants and their leaf areas recorded using the LI-3000 portable leaf area meter. The leaves of each plant were placed in a labelled paper bag The stems and below surface portion of four replicates of each species were carefully removed from the PVC tubes and washed with water to remove any sand adhering to the roots These stems and below surface parts of plants were then gently blotted with paper towels and placed in paper bags containing the leaves of the same plants

The above ground stems of the remaining four replicates were also removed and placed in paper bags containing their respective leaves. There were four replicates of each treatment for each species that had the complete plant and four replicates that had only the leaves and the stems because their roots were used to assess VAM colonization. These plant materials were then dried in an oven at 70°C for 48 hours and weighted.

5.2.2.4 Assessment of root colonization and plant nutrient analysis

The roots of plants from four replicates were examined for mycorrhizal root colonization. In the buried treatments VCS/VFS (+-) and VFS/VCS (-+) where both the mycorrhizae and non-mycorrhizal sand was used for burial, care was taken to keep plant parts above and below the burial surface separated to avoid contamination. The roots were then cleared and stained following Brundrett *et al.*, (1984). Assessment of mycorrhizal colonization of roots was done using the magnified intersections method (McGonigle *et al.*, 1990).

After recording the biomass, the plant material for each replicate was finely ground by using a Wiley mill and then analyzed for nitrogen, phosphorus and potassium at the analytical services laboratory, University of Guelph, Ontario. Four samples of the sand that had the mycorrhizae and sand without mycorrhizae were also submitted for analysis to determine differences if any in the nutrient levels.

5.2.3 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the data for carbon dioxide exchange rate (CER), photochemical efficiency (F_v/F_m), plant height, leaf area, biomass, root colonization and plant nutrient content to determine differences between the treatments. This was followed by Bartlett's test for equality of variances. If the ANOVA results produced significant F-values, Tukey's test was applied to find specific differences between treatments by multiple comparison tests. Both analyses were performed at 0.05 significance level.

5.3 Results

5.3.1 Survey of VAM Colonization

The techniques used for clearing and staining of roots were very effective At high magnification (200x) the arbuscules, vesicles and hyphae were clearly distinguishable (Plates 5.3 and 5.4). The survey of vesicular-arbuscular mycorrhizal (VAM) colonization in sand dune plants indicated that VAM were ubiquitous among dune plants of the twelve species tested. *Equisetum arvense* was the only non-mycorrhizal plants. The roots of *Cakile edentula* and *Corispermum hyssopifolium* had very low levels (4-6%) of arbuscular and hyphal colonization (Table 5.1). The other nine species contained both vesicules and arbuscules, although *Ammophila breviligulata* had 98% arbuscular and only 1% vesicular colonization *Agropyron psammophilum, Elymus canadensis, Melilotus alba, Oenothera biennis, Panicum virgatum* and *Tusilago farfara* also had fairly high (25-45%) arbuscular colonization (Table 5.1). In general, all the three annuals and one biennial had low vesicular and arbuscular colonization.

The vesicular colonization of VAM containing species were rather substantial and ranged from 8% in *Tusilago farfara* to 45% in *Melilotus alba. Cakile edentula* and *Corispermum hyssopifolium*, the two arbuscular mycorrhizal species, had the lowest (approx 5%) arbuscular colonization. They also had very low hyphal colonization (Table 5.1). Hyphal colonization was fairly high (51 to 98%) in all other VAM containing species.

5.3.2 Greenhouse Burial Experiment

5.3.2.1 Carbon dioxide Exchange Rate

The mean carbon dioxide exchange rate showed a significant increase in plants growing in sterile soil infected with mycorrhizae (+) as compared to those growing in sterile soil with no mycorrhizae (-) Both treatments of *A psammophilum* and *P. virgatum* served as controls for buried treatments (Fig. 5.1). There was a significant

Plate 5.3 VAM colonization of *Xanthium strumarium* roots collected from Port Burwell Provincial Park in October, 1992.

Photographs a-d show various fungal structures; a=arbuscules; v=vesicles; h=hyphae

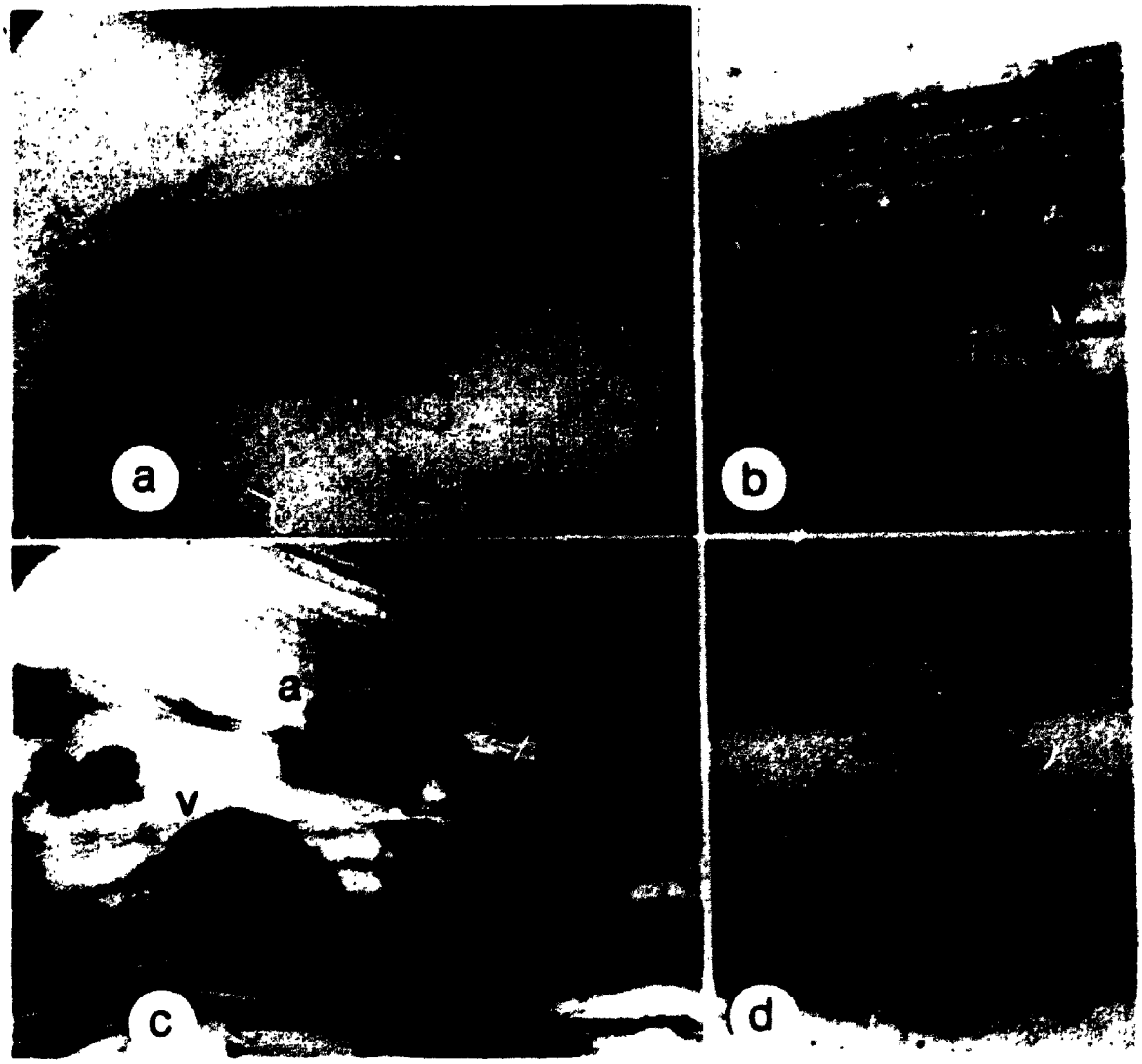

a
b
a
v
c
d

Plate 5.4 VAM colonization of *Panicum virgatum* roots in the greenhouse burial experiment.

Photographs a and b show various fungal structures; a=arbuscules; v=vesicles; h=hyphae.


a
a

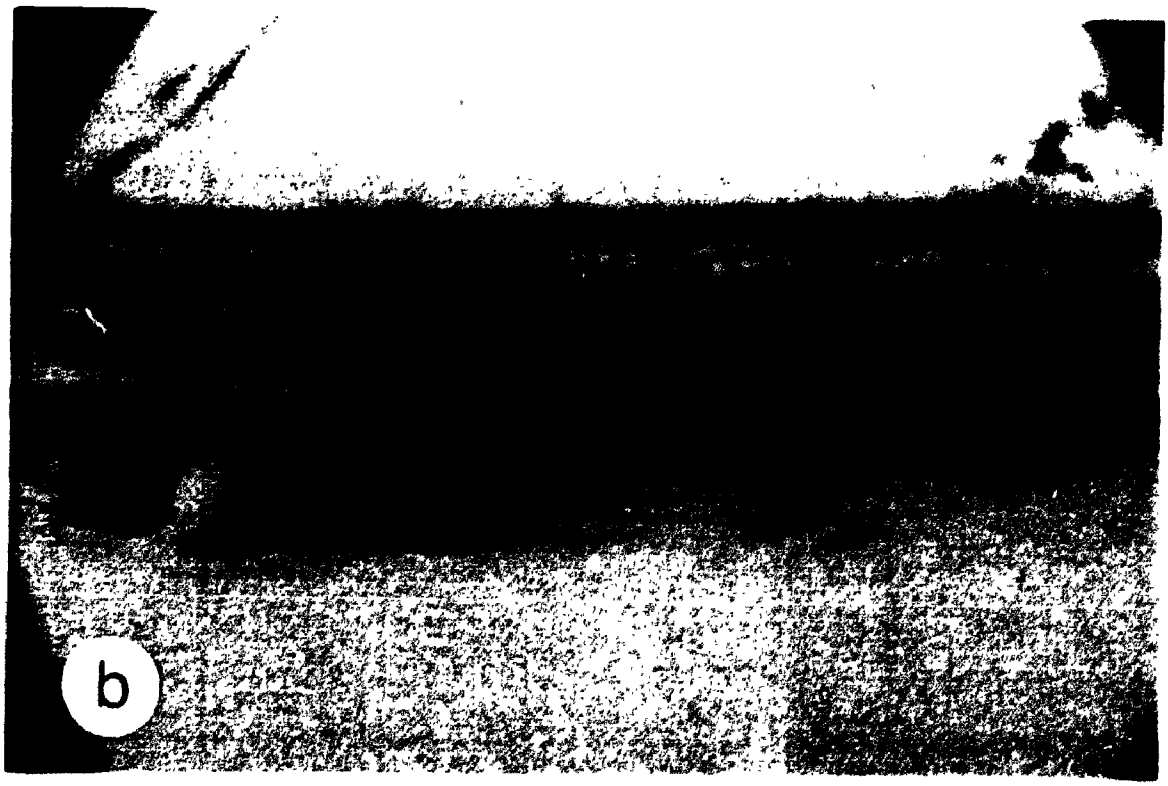
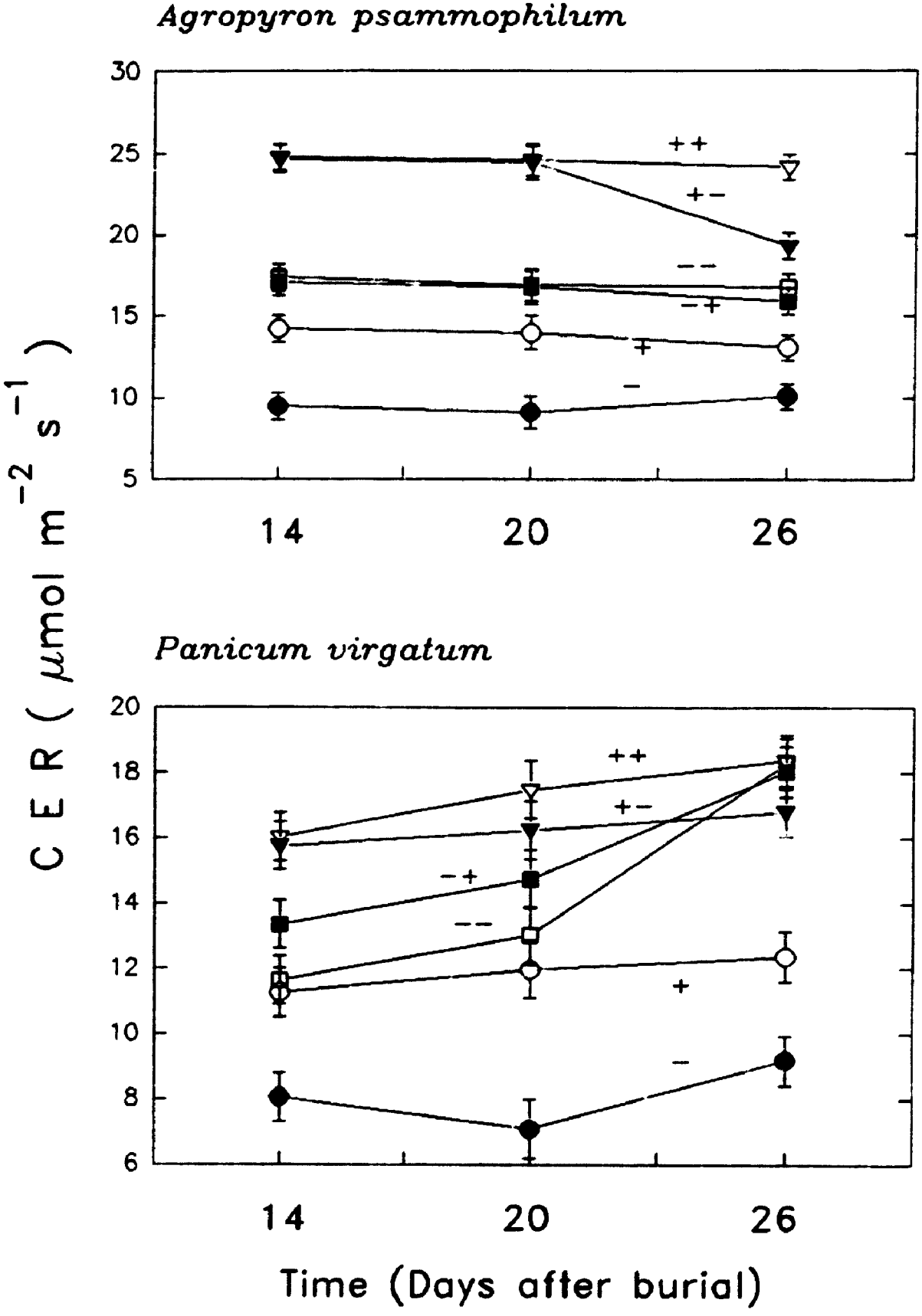

b

Table 5.1: Mean (%) and one standard error (SE) of arbuscular (AC), vesicular (VC), and hyphal colonization (HC) of twelve common dune species collected from Port Burwell Provincial Park. NC = No colonization.

Species	AC		VC		HC	
	mean	SE	mean	SE	mean	SE
Agropyron psammophilum	37.3	±2.30	18.3	±2.30	94.1	±3.21
Ammophila breviligulata	98.1	±2.88	1.4	±0.57	52.2	±2.89
Cakile edentula	4.4	±1.15	NC		7.0	±1.53
Corispermum hyssopifolium	6.3	±1.29	NC		11.3	±1.26
Elymus canadensis	36.0	±3.21	32.1	±2.00	98.2	±3.51
Equisetum arvense	NC		NC		NC	
Melilotus alba	31.4	±3.20	45.2	±2.51	88.1	±1.00
Oenothera biennis	39.2	±2.13	13.3	±2.52	85.3	±2.08
Panicum virgatum	45.1	±3.62	12.4	±4.04	83.0	±3.56
Strophostyles helvola	17.0	±1.83	28.2	±4.51	77.4	±4.65
Tusilago farfara	25.3	±6.03	8.4	±3.21	84.2	±2.32
Xanthium strumarium	14.2	±3.78	6.3	±0.57	51.2	±2.08

Figure 5.1 Mean (± SE) carbon dioxide exchange rate (CER) of *Agropyron psammophilum* and *Panicum virgatum* plants buried or unburied with sterilized or sterilized mycorrhizae containing sand. Plants grown in sterilized soil plus mycorrhizae (+,), sterilized soil minus mycorrhizae (-,●); sterile soil plus mycorrhizae and buried with sterile soil plus mycorrhizae (++,▽), sterile soil plus mycorrhizae and buried with sterile soil minus mycorrhizae (+ -,▼); sterile soil minus mycorrhizae and buried with sterile soil plus mycorrhizae (- +, □); sterile soil minus mycorrhizae and buried with sterile soil minus mycorrhizae (--, ●).


Agropyron psammophilum
30
25
20
15
10
5
++
+−
−−
−+
+
−
Panicum virgatum
20
18
16
14
12
10
8
6
++
+−
−+
−−
+
−
14
20
26
CER (μmol m⁻² s⁻¹)
Time (Days after burial)

($P<0.001$) difference in the final CER readings between the unburied controls (VCS/VFS +-) and the buried plants (VCS/VCS ++) and (VCS/VFS +-) of *A. psammophilum* and *P. virgatum* (Table 5.2).

There was no difference in the CER between the mycorrhizae-containing buried plants (VCS/VFS +-) and those planted in non-mycorrhizae containing sand (VFS/VFS -- and VFS/VCS -+) of *A. psammophilum* and *P. virgatum* (Table 5.2). However, there were significant differences at the 3rd CER reading (26 days after burial) between *A. psammophilum* plants buried with sand containing mycorrhizae (VCS/VCS ++) and that buried with sand without mycorrhizae (VCS/VFS +-). In *P. virgatum* there was a significant difference between CER readings for VFS/VFS (--) and VFS/VCS (+-) treatments taken at 20 and 26 days after burial (Fig.5.1).

5.3.2.2 Photochemical Efficiency

The F_v/F_m of *A. psammophilum* and *P. virgatum* taken over time (14, 20 and 26 days) are presented in Fig. 5.2 and the final readings are given in Table 5.3. The data for unburied control plants showed a significant increase in photochemical efficiency in both *A. psammophilum* and *P. virgatum* growing in the mycorrhizae-containing sand compared to those growing in mycorrhizae free sand (Fig. 5.2). The four burial treatments of *P. virgatum* (VCS/VCS ++, VCS/VFS +-, VFS/VFS --, and VFS/VCS -+) showed a significant increase in the F_v/F_m readings between 20 and 26 days after burial (Fig. 5.2). There was a significant ($P<0.001$) difference in the final F_v/F_m readings between *A. psammophilum* plants growing in the unburied VAM free sand (VFS -) and those buried in the VAM containing sand (VCS/VCS ++) (Table 5.3). No other treatments of *A. psammophilum* showed any significant differences. In *P. virgatum* there was a significant difference between the unburied (VCS + and VFS -) and the buried (VCS/VCS ++, VCS/VFS +-, VFS/VFS --, and VFS/VCS -+) plants (Table 5.3). A significant difference was also observed between plants in the unburied mycorrhizae-containing (VCS) sand and the unburied VAM free sand (VFS) (Table 5.3).

Table 5.2: Mean (%) and one standard error (SE) for Carbon dioxide exchange rate (μ mol m^{-2} s^{-1}) of *Agropyron psammophilum* and *Panicum virgatum* recorded five weeks after burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

	A. psammophilum		*P. virgatum*	
	mean	SE	mean	SE
VCS (+)	13.1 ab	0.6	12.4 a	0.7
VFS (–)	10.1 a	0.5	9.2 a	0.5
VCS/VCS (+ +)	24.2 d	1.4	18.4 b	1.0
VCS/VFS (+ –)	19.3 c	0.6	16.8 b	1.0
VFS/VFS (– –)	16.8 c	0.8	18.3 b	0.5
VFS/VCS (– +)	15.9 bc	0.4	18.1 b	0.8

Figure 5.2 Mean (± SE) photochemical efficiency (F_v/F_m) of *Agropyron psammophilum* and *Panicum virgatum* plants buried or unburied with sterilized or sterilized plus mycorrhizae containing sand. Plants grown in sterilized soil plus mycorrhizae (+, ○); sterilized soil minus mycorrhizae (-,●); sterile soil plus mycorrhizae and buried with sterile soil plus mycorrhizae (++,▽), sterile soil plus mycorrhizae and buried with sterile soil minus mycorrhizae (+-,▼); sterile soil minus mycorrhizae and buried with sterile soil plus mycorrhizae (-+, □); sterile soil minus mycorrhizae and buried with sterile soil minus mycorrhizae (--, ■).

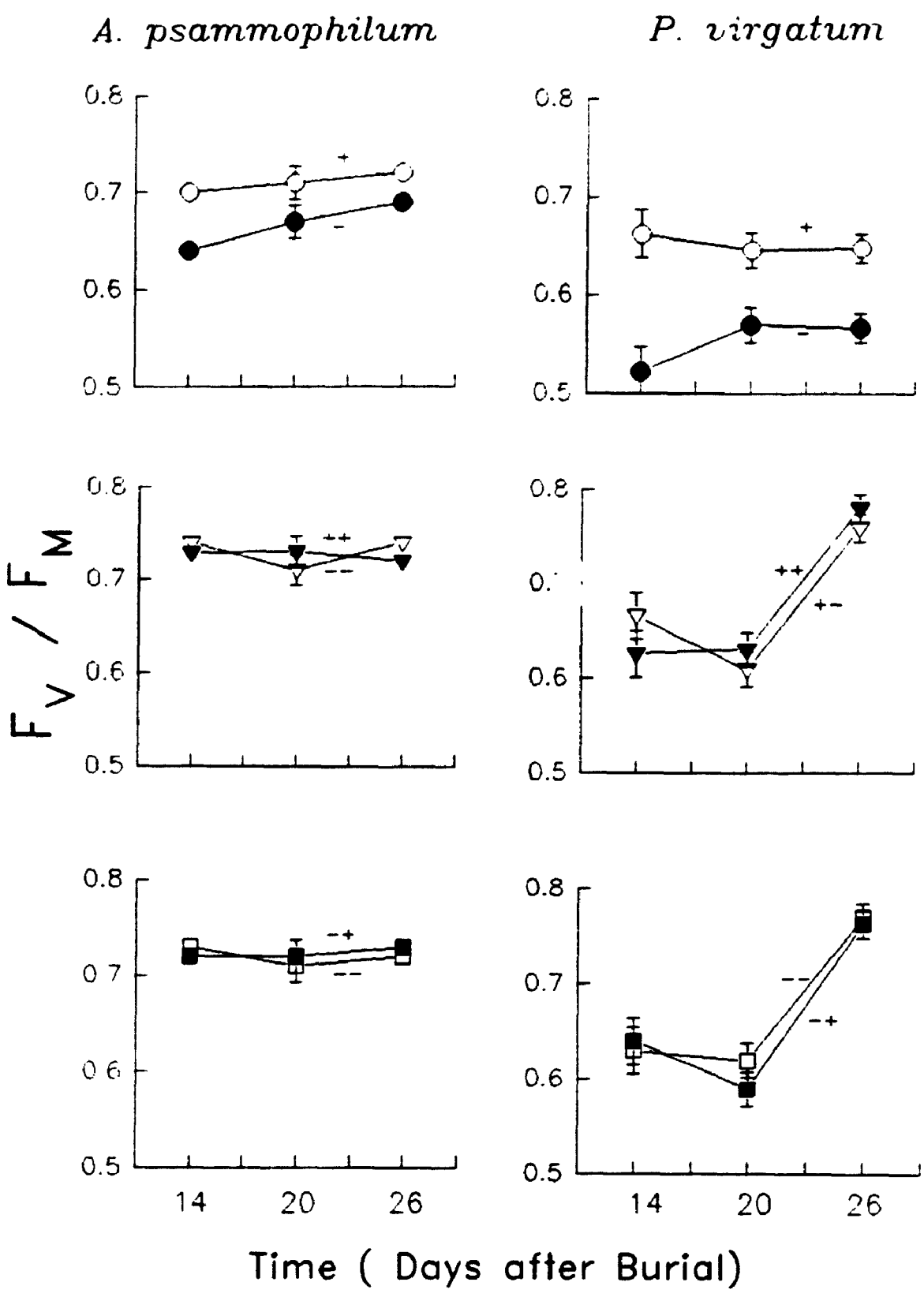

A. psammophilum
P. virgatum
F_V / F_M
0.8
0.7
0.6
0.5
+
−
++
−−
−+
14
20
26
Time (Days after Burial)

Table 5.3: Mean and one standard error (SE) for photochemical efficiency (F_v / F_m) of *Agropyron psammophilum* and *Panicum virgatum* recorded five weeks after burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

	A. psammophilum		*P. virgatum*	
	mean	SE	mean	SE
VCS (+)	0.72 ab	0.01	0.65 b	0.01
VFS (–)	0.69 a	0.01	0.57 a	0.02
VCS/VCS (+ +)	0.74 b	0.01	0.76 c	0.01
VCS/VFS (+ –)	0.72 ab	0.12	0.78 c	0.02
VFS/VFS (– –)	0.72 ab	0.01	0.77 c	0.01
VFS/VCS (– +)	0.73 ab	0.01	0.76 c	0.02

5.3.2.3 Plant height, leaf area and total biomass

Plant Height

The mean plant height data for *A. psammophilum* and *P. virgatum* are presented in Table 5.4. In both species there were no significant differences between unburied plants growing in the mycorrhizal (VCS) and non mycorrhizal (VFS) sand (Table 5.4). In *A. psammophilum* burial of plants significantly increased plant height as compared to unburied plants. Plants grown in mycorrhizae containing sand and then buried in VAM containing or VAM free sand were significantly taller than those planted in VAM free sand and then buried in mycorrhizal or non-mycorrhizal sand (Table 5.4). If the plants were growing in sterile sand with mycorrhizae, they showed an increase in plant height irrespective of the burial medium (VAM free or VAM containing sand) used for the burial treatment.

In *P. virgatum* there was a significant difference between the unburied controls (VCS + and VFS -) and all burial treatments (VCS/VCS ++, VCS/VFS +-, VFS/VFS --, VFS/VCS -+) (Table 5.4). However, no significant differences were obtained within the burial treatments.

Leaf Area

There was no significant difference in the leaf area of the mycorrhizal (VCS +) and non-mycorrhizal (VFS -) controls of *A. psammophilum*, however, there was a significant difference between the VCS and VFS of *P. virgatum* (Table 5.5).

Burial of plants stimulated the growth of leaves as shown by a significant increase in leaf area of buried plants of *A. psammophilum*. A significant difference was also seen between plants grown in mycorrhizae-containing sand and then buried with VAM containing (VCS/VCS ++) or VAM free sand (VCS/VFS +-) and those grown in mycorrhizae-free sand and then buried with VAM free (VFS/VFS --) or VAM containing sand (VFS/VCS -+).

In *P. virgatum* all plants grown in VAM containing sand produced significantly greater leaf area than all those grown in VAM free sand (Table 5.5). Sand used for burial (VAM free or VAM containing) did not make any difference.

Total Dry Biomass

The means of the total dry biomass of *A. psammophilum* and *P. virgatum* are

Table 5.4: Mean (%) and one standard error (SE) for plant height (cm) of *Agropyron psammophilum* and *Panicum virgatum* recorded five weeks after burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

	A. psammophilum		*P. virgatum*	
	mean	SE	mean	SE
VCS (+)	20.15 a	0.47	13.80 a	0.37
VFS (–)	19.13 a	0.31	12.05 a	0.74
VCS/VCS (+ +)	40.25 c	1.59	22.68 b	0.85
VCS/VFS (+ –)	42.33 c	1.91	22.85 b	0.78
VFS/VFS (– –)	33.00 b	0.98	20.35 b	1.07
VFS/VCS (– +)	32.30 b	1.90	18.80 b	0.67

Table 5.5: Mean (%) and and standard error (SE) for leaf area (cm^2) of *Agropyron psammophilum* and *Panicum virgatum* recorded five weeks after burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

	A. psammophilum		*P. virgatum*	
	mean	SE	mean	SE
VCS (+)	7.69 a	0.48	5.77 b	0.14
VFS (–)	6.53 a	0.53	3.75 a	0.17
VCS/VCS (+ +)	17.64 c	0.75	7.56 c	0.21
VCS/VFS (+ –)	17.91 c	0.64	7.14 c	0.55
VFS/VFS (– –)	13.96 b	0.70	3.88 a	0.16
VFS/VCS (– +)	14.46 b	0.88	4.11 a	0.25

presented in Table 5 6 In both species there was no significant difference between control plants grown in VAM free (VFS -) or VAM containing sand (VCS +)

In *A psammophilum* the plants grown in mycorrhizae-containing sand (VCS/VCS ++ and VCS/VFS +-) produced significantly higher biomass than the unburied control (VCS + and VFS -) and plants buried in VAM free sand with VAM free sand (VFS/VFS --) and VAM free sand buried with VAM containing sand (VFS/VCS -+) (Table 5.6)

In *P. virgatum* there was a significant difference between the unburied controls (VCS and VFS) and the burial treatments (VCS/VCS ++, VCS/VFS +- and VFS/VFS --), but there was no significant difference between the plants grown in VAM containing sand and those grown in VAM free sand and then buried in VAM containing sand (VFS/VCS -+) (Table 5 6)

5.3.2.4 Root colonization assessment and plant nutrient analysis

The results of the mycorrhizal colonization of roots are presented in Table 5 7 No mycorrhizal colonization was observed in the VFS (-), VFS/VFS (--) and VFS/VCS (-+) treatments confirming that in sterilized soil VAM had been killed and there was no contamination in the experiment. The percentages of arbuscular (AC) and vesicular (VC) colonization were slightly higher in the VAM containing control (VCS +) but were not significantly different from the VCS/VCS (++) and VCS/VFS (+-) treatments *of A psammophilum* (Table 5.7). In *P. virgatum* the vesicular colonization was significantly higher in the VCS (+) than in the VCS/VCS (++) and VCS/VFS (+-) treatments (Table 5.7).

The percent nitrogen, phosphorus and potassium in plants growing in control and treated plots are presented in Table 5.8. There was no significant difference in the percentage of nitrogen and phosphorus between any of the treatments for *A psammophilum* plants (Table 5 8). However there was a significant difference between the percentage of potassium between the unburied and buried plants of *A. psammophilum* grown in mycorrhizae-free sand In *P. virgatum* there was no significant difference in the percentage of potassium between the different treatments The percentage of nitrogen and potassium in *P virgatum* had some significant differences between the unburied

Table 5.6: Mean and one standard error (SE) for total plant biomass (gms) of *Agropyron psammophilum* and *Panicum virgatum* recorded five weeks after burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

	A. psammophilum		*P. virgatum*	
	mean	SE	mean	SE
VCS (+)	4.73 a	0.23	3.09 ab	0.12
VFS (–)	4.02 a	0.31	2.48 a	0.14
VCS/VCS (++)	6.87 b	0.26	5.15 c	0.26
VCS/VFS (+–)	6.98 b	0.42	4.98 c	0.40
VFS/VFS (––)	5.08 a	0.13	4.60 c	0.22
VFS/VCS (–+)	5.11 a	0.10	4.21 bc	0.39

Table 5.7: Mean (%) and one standard error (SE) for arbuscular (AC), vesicular (VC), and hyphal colonization (HC) of *Agropyron psammophilum* and *Panicum virgatum* after five weeks of burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

Table 5.7:

		AC		VC		HC	
		mean	SE	mean	SE	mean	SE
A. psammophilum	VCS (+)	0.11 b	0.17	0.10 b	0.03	0.22 b	0.04
	VFS (–)	0.0 a		0.0 a		0.0 a	
	VCS/VCS (+ +)	0.09 b	0.02	0.05 ab	0.01	0.20 b	0.03
	VCS/VFS (+ –)	0.05 ab	0.01	0.04 ab	0.01	0.14 b	0.03
	VFS/VFS (– –)	0.0 a		0.0 a		0.0 a	
	VFS/VCS (– +)	0.0 a		0.0 a		0.0 a	
P. virgatum	VCS (+)	0.31 b	0.04	0.12 c	0.02	0.68 c	0.07
	VFS (–)	0.0 a		0.0 a		0.0 a	
	VCS/VCS (+ +)	0.27 ab	0.03	0.06 b	0.01	0.50 b	0.04
	VCS/VFS (+ –)	0.17 ab	0.20	0.45 ab	0.01	0.37 b	0.06
	VFS/VFS (– –)	0.0 a		0.0 a		0.0 a	
	VFS/VCS (– +)	0.0 a		0.0 a		0.0 a	

Table 5.8: Mean and one standard error (SE) for percent nitrogen, phosphorus, and potassium in *Agropyron psammophilum* and *Panicum virgatum* plants after five weeks of burial. For this experiment sterilized sand was used for all treatments. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test. Percentages were transformed to Arcsine $\sqrt{}$ before data analysis.

Table 5.8:

		% N		% P		% K	
		mean	SE	mean	SE	mean	SE
A. psammophilum	VCS (+)	1.12 a	0.15	0.20 a	0.02	1.21 a	0.15
	VFS (–)	1.25 a	0.07	0.21 a	0.01	1.26 a	0.15
	VCS/VCS (++)	1.28 a	0.08	0.21 a	0.01	1.62 ab	0.07
	VCS/VFS (+–)	1.14 a	0.05	0.19 a	0.01	1.58 ab	0.08
	VFS/VFS (––)	1.17 a	0.03	0.20 a	0.01	1.76 b	0.04
	VFS/VCS (–+)	1.11	0.04	0.19 a	0.01	1.71 b	0.04
P. virgatum	VCS (+)	1.24 a	0.08	0.20 a	0.01	1.02 a	0.05
	VFS (–)	1.34 ab	0.05	0.22 ab	0.01	1.05 a	0.03
	VCS/VCS (++)	1.42 abc	0.02	0.23 abc	0.01	1.08 a	0.03
	VCS/VFS (+–)	1.55 bc	0.07	0.23 abc	0.01	1.13 a	0.02
	VFS/VFS (––)	1.62 c	0.05	0.24 bc	0.01	1.08 a	0.11
	VFS/VCS (–+)	1.66 c	0.07	0.25 c	0.01	1.14 a	0.21

Table 5.9: Mean and one standard error (SE) for total nitrogen, phosphorus, and potassium (in grams) in *Agropyron psammophilum* and *Panicum virgatum* plants after five weeks of burial. For this experiment sterilized sand was used for each treatment. VCS = VAM containing sand, no burial; VFS = VAM free sand, no burial; VCS/VCS = VAM containing sand, buried with VAM containing sand; VCS/VFS = VAM containing sand, buried with VAM free sand; VFS/VFS = VAM free sand, buried with VAM free sand; VFS/VCS = VAM free sand, buried with VAM containing sand. Means followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

Table 5.9:

		Total N (g)		Total P (g)		Total K (g)	
		mean	SE	mean	SE	mean	SE
A. psammophilum	VCS (+)	49.27 a	6.7	8.60 a	1.1	53.47 a	6.5
	VFS (–)	54.46 ab	2.9	9.12 ab	0.3	55.14 b	6.4
	VCS/VCS (+ +)	88.33 c	7.7	14.36 c	0.9	111.80 b	7.9
	VCS/VFS (+ –)	78.66 c	3.3	12.88 c	0.5	109.71 b	8.4
	VFS/VFS (– –)	74.68 bc	1.8	12.46 c	0.7	112.49 b	2.3
	VFS/VCS (– +)	70.25 abc	2.2	11.94 bc	0.4	108.90 b	2.0
P. virgatum	VCS (+)	53.59 a	3.5	8.59 a	0.5	43.98 a	2.0
	VFS (–)	57.28 a	1.9	9.23 a	0.4	44.67 a	1.0
	VCS/VCS (+ +)	90.47 b	1.0	14.53 b	0.5	68.90 b	2.2
	VCS/VFS (+ –)	100.90 b	4.8	15.02 b	0.5	73.54 b	2.5
	VFS/VFS (– –)	102.97 b	3.5	15.37 b	0.4	68.78 b	2.2
	VFS/VCS (– +)	103.16 b	5.8	15.71 b	0.6	70.52 b	2.0

mycorrhizae-containing plants (VCS +) and the buried non-mycorrhizae containing (VFS/VFS -- and VFS/VCS -+) plants (Table 5.8).

In *A. psammophilum* the total nitrogen content of the unburied plants growing in VAM containing soil (VCS +) was significantly lower than the buried plants (VCS/VCS ++, VCS/VFS +- and VFS/VFS --) (Table 5.9). There was also a significant increase in nitrogen between unburied mycorrhizae-free (VFS -) and buried mycorrhizae-containing (VCS/VCS ++ and VCS/VFS +-) plants of *A. psammophilum*. The total phosphorus content of unburied mycorrhizae-containing plants of *A. psammophilum* were significantly lower than all the buried (VCS/VCS ++, VCS/VFS +-, VFS/VFS -- and VFS/VCS -+) plants (Table 5.9). In the case of potassium there was a significant difference between the unburied mycorrhizae-containing control plants and all other treatments

In *P. virgatum* there was no significant difference between the two unburied treatments (VCS + and VFS -) in the total nutrient content in any of the three elements (NPK). However, there was a significant increase between the unburied control treatments (VCS + and VFS -) and all the buried (VCS/VCS ++, VCS/VFS +-, VFS/VFS --, VFS/VCS -+) treatments for all three elements, nitrogen, phosphorus and potassium (Table 5.9)

5.4 Discussion

Mycorrhizal fungi are ubiquitous in sand dune systems. The majority of sand dune species tested had mycorrhizae associated with their root systems. However, the extent of colonization and the ratio between arbuscules and vesicles varied among species depending on the life form of the species and the length of growing season. Since vesicles are storage sites and arbuscules are the sites of nutrient exchange (Hirrel *et al.*, 1978), the number of these structures would likely depend on the growth stage of the plant.

A close comparison between life forms of dune plants showed that the perennials and biennials had a higher percentage of colonization than the annuals. This could be due to the carryover of colonization from the previous growing seasons in

perennials and biennials and short length of the growing season in the annuals. Perhaps the date of sample collection (September) may have been too late because the annuals had started to senesce by that time. Moreover, the infection of annuals by mycorrhizal fungi would have to start after the germination of seeds in early spring and would depend on the amount of inoculum present in the soil, in the vicinity of a seedling. The absence or low degree of colonization may also be explained by the ability of dune annuals to germinate very early in spring, when VAM development is low owing to their sensitivity to spore germination and infection at low temperatures (Sanders *et al.*, 1977; Black and Tinker, 1979; Tommerup, 1983).

The absence of mycorrhizae in *Equisetum arvense* agrees with findings of earlier researchers who classified the genus as non-mycotrophic (Ocampo *et al.*, 1980; Berch and Kendrick, 1982). In recent years the mycorrhizal status of *Equisetum* species had been somewhat controversial (Dhillion, 1993). Lohman (in Dhillion, 1993) had reported the occurrence of an endophytic 'phycomycete' in *Equisetum arvense*. But other workers have reported the absence of endophytes in Equisetae (Dhillion, 1993). Koske *et al.*, (1985) pointed out that these apparently non-mycorrhizal species may have been collected from wet habitats, where the high substrate moisture decreases mycorrhizal establishment. The samples of *Equisetum arvense* collected in this study were also growing in a wet slack. Therefore the lack of VAM colonization may also be due to the moist substrate (Koske *et al.*, 1985).

The presence of vesicles and hyphae in the roots of *Cakile edentula* (Brassicaceae) and *Corispermum hyssopifolium* (Chenopodiaceae) observed in this study disagrees with reports of early investigators such as Stahl (1900); Asai (1934) and Maeda (1954)(cited in Hirrel *et al.*, 1978). Their studies had indicated that Chenopodiaceae and Brassicaceae were non-mycorrhizal. However, our results agree with studies where some members of these families may contain low or in some instances high levels of vesicular-arbuscular (VA) mycorrhizal infection (Hirrel *et al.*, 1978).

The high percent colonization of *Ammophila breviligulata* could probably explain why this species is the most successful colonizer of many dune systems. The results are consistent with percentages reported for some of these species and other perennial grasses in dune communities (Giovannetti and Nicolson, 1983; Forster and

Nicolson, 1981, Koske and Halvorson, 1981)

The hypothesis that the association of mycorrhizal fungi enhances growth of sand dune species was partially accepted. The carbon dioxide exchange rate (CER) and the photochemical efficiency (F_v/F_m) data revealed that mycorrhizae do play an important role in the growth of buried plants. Although the differences between mycorrhizal and non-mycorrhizal buried plants were not always significant, there was a clear trend towards a positive role played by mycorrhizae f growth

Although mycorrhizae played a significant role in improving the growth and vigour of buried plants, it alone was not responsible for the growth enhancement shown by plants in the burial treatments. The stimulated growth exhibited by partially buried plants may be due to a number of other factors. For example, sand burial may, (i) protect the root system of plants from drying out (McLeod and Murphy, 1977, Zhang and Maun, 1991), (ii) provide increased nutrient input and increased rooting surface for root expansion of plants (Baldwin and Maun, 1988; Hawke and Maun, 1988); (iii) replace the substrate containing injurious soil microorganisms with fresh sand without harmful microorganisms (van der Putten, (1989); (iv) lower interspecific competition through the elimination of plants intolerant of sand accretion (Huiskes, 1979; Disraeli, 1984); and (v) enhance net CO_2 assimilation (Yuan *et al.*, 1993). The mycorrhizae seemed to be partly responsible for the enhanced growth following burial of dune plants. Morris *et al.*, 1974 also showed that sand burial improved symbiotic nitrogen fixation and mycorrhizal fungi in the root of buried plants. The absence of VAM colonization of plants grown in VAM free sterilized soil and buried with VAM containing sand could be due to the short period of time during which the new roots were exposed to the VAM inoculum. Preliminary experiments on root development showed that in buried plants, the new roots do not initiate until about 10-15 days after burial depending on the type of species and the time (season) of the year. Since the plants were harvested 4 weeks after burial in this experiment, the extent of colonization was generally low and newly buried plants were not colonized. Also, the percent colonization was lower for the buried plants as compared to control because there were more new uncolonized roots in the buried parts of the plants

The presence of mycorrhizal hyphae and associated plant roots not only increase the nutrient status and water uptake of plants but also change root exudates, the rhizosphere biota and their influence on plant growth (Allen *et al*, 1979) Since the amount of exudates and the level of infection of roots may differ from species to species, the role of mycorrhizal fungi would be variable for different species. That may be why the two species, *Agropyron psammophilum* and *Panicum virgatum* responded differently to mycorrhizal treatments.

CHAPTER SIX

GENERAL CONCLUSIONS

Plants growing in the foredune and strandline habitats face a number of environmental stresses, chief among which is sand accretion. Many sand dune species are well adapted to withstand this stress and will emerge above the new sand surface through the growth of rhizomes, suckers, stems, hypocotyls, petioles, and other structures (Sykes and Wilson, 1990) Some plants have become so specialized that they require regular burial in sand to maintain high vigour (Eldred and Maun, 1982). The role of VAM in sand dunes, both in binding sand grains into stable aggregates and in providing nutrients, has been suggested by several researchers (Koske, 1975; Nicolson, 1959; Sutton and Sheppard, 1976)

In this thesis I have examined the effects of sand accretion on (i) the distribution and composition of sand dune communities, (ii) the physiological ecology of annual, biennial and perennial plant species and (iii) the role of vesicular-arbuscular mycorrhizal fungi (VAM) on the enhancement of growth in buried plants. We will examine each of the three aspects separately

Sand accretion acts as a very strong selective force in altering the composition and density of vegetation by selective elimination of intolerant species and increasing the relative abundance of tolerant species (Eldred and Maun, 1982). Moreno-Casasola (1985) suggested that there may be a correlation between the amount of sand movement and plant community structure. The first hypothesis that plant species are distributed in the foredunes according to their ability to withstand sand deposition was accepted The data clearly showed that sand movement (deposition and erosion) was variable in different microsites and depended on the distance from the lake, type of vegetation, amount of available sand and the time of the year. Based on this study the dune species may be grouped into 3 categories on the basis of their tolerance to sand accretion First category consists of foredune perennial grasses namely, *Ammophila breviligulata*, *Calamovilfa longifolia*, *Agropyron psammophilum* and *Panicum virgatum* which can withstand high amounts of sand deposition ranging between 40 and 80 cm

annually These perennial grasses have large amount of storage in their roots and rhizomes that facilitate the growth and emergence of shoots from burial depths. A number of factors including root depth, lower competition, added nutrients and greater aeration probably result as beneficial effects of sand deposition in these species. Their ability to rapidly increase in height when buried coupled with increased space for root growth, more nutrients in the deposited sand and other benefits allows these perennial grasses to grow well in areas of high sand deposition (Eldred and Maun, 1982; Harris and Davy, 1986; Sykes and Wilson, 1990).

The second group comprises monocarpic perennials such as *Artemisia campestris*, *Melilotus alba*, *Xanthium strumarium* and an annual *Strophostyles helvola*, which can tolerate medium amounts of sand deposition ranging between 10 and 20 cm annually. The monocarpic perennials do not possess the extensive roots and rhizomes present in perennial grasses; however they have large tap roots with stored reserves especially in the second year of their growth cycle. *Strophostyles helvola* is a robust annual legume with fast rate of growth and considerable powers of lateral root development and nodule formation.

The third catagory consists of annual species such as *Cakile edentula*, *Corispermum hyssopifolium* and *Euphorbia polygonifolia* which can withstand small amounts of sand deposition ranging between 5 and 10 cm annually. These plants are found on the beach where they may be completely or partially buried by sand. This study showed that complete burial killed all plants. Since these species produce large numbers of seedling in spring the buried seedlings die and hence the seedling population is reduced.

The second hypothesis that burial in sand stimulates plant growth through an increase in photosynthetic capacity and photochemical efficiency was also accepted. Experiments conducted in the field, greenhouse and growth chamber clearly showed that photosynthetic capacity (CER), photochemical efficiency (F_v/F_m), leaf area, leaf thickness, biomass, root/shoot ratio, chlorophyll a/b ratio and total chlorophyll content increased in buried treatments as compared to control. Minor discrepancies were evident especially in the field experiment where the environment was variable and controlled. The results of this study agree with those of Yuan *et al.*, (1993) who

recorded an increase in CO_2 uptake, biomass, leaf area and leat thickness in partially buried *Ammophila breviligulata* and *Calamovilfa longifolia* plants. The buried plants had a higher CER than control plants probably because of greater root depth, lowered competition and greater availability of nutrients

The third hypothesis that VA-mycorrhizae improves the vigour and total biomass of plants was accepted. The survey revealed that a large majority of tested species (11 out of 12) were colonized by VA-mycorrhizae. Mycorrhizal hyphae also formed aggregates of bound sand grains which may be of considerable importance in sand dune stabilization (Koske and Halvorson, 1980). Although VA-mycorrhizae enhanced the morphological and physiological responses of dune plants, they were not solely responsible for the enhanced growth of buried plants. It was observed in this study and also evidenced in other studies that buried portions of the stem produces new roots and root hairs, which are very quickly colonized by VA-mycorrhizae. Plants growing in soil infected with mycorrhizal fungi contained larger amounts of phosphorus which is limiting in the dunes and thus the mycorrhizae were able to mobilize the phosphorus contained in the sand deposits. Further research is needed to examine the interactions between VA-mycorrhizae, and other soil organisms and plants.

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