The anatomy and ecophysiology of *Mariscus congestus* from three different habitats in the Albany and Bathurst districts of the Eastern Cape, investigated under field and laboratory conditions

THESIS

Submitted in fulfilment of the requirements

the degree of

Master of Science

RHODES UNIVERSITY

by

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January 1992

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ABSTRACT

An investigation of the anatomy and gas exchange characteristics of *Mariscus congestus* in three different habitats was undertaken in order to establish whether *M. congestus* from the three different habitats displayed any ecotypic responses when placed in a new similar environment. It was hoped that the results of this investigation would yield evidence that would support the ecotype concept similar to the investigations of Milner and Hiesey (1964), Green (1969) and Slayter and Ferrar (1977).

On the basis of the site leaf anatomy, *M. congestus* investigated at the coast (site 1) differed in many respects from the inland plants (sites 2 and 3). These differences suggest that the coastal plants may have undergone a slight ecotypic divergence from the inland plants. The anatomical investigation also suggested that the leaves of *M. congestus* from all three sites may either be C_4 NADP-ME or PCK and that all had typical Chlorocyperiod anatomy.

The habitat microclimates at sites 1-3 had different light and water regimes. There were no significant differences between the 12 month temperature environments of the three sites. There was however, a minor difference between the coastal (high temperature) and the inland (lower temperature) sites. *M. congestus* at the three sites had significantly different CO_2 assimilation rates, transpiration and stomatal conductance in response to the differing habitat microclimates. The water use efficiency of the sites were however, similar. Site 1 attained the highest CO_2 assimilation rates, transpiration, stomatal conductance, and water use efficiency and site 3 the lowest.

Under similar conditions the gas exchange data for the potted plants indicated that *M. congestus* from the different sites was typically C_4. The optimal photosynthetic temperatures of all the sites was above 30°C and they did not show significant inhibition of CO_2 assimilation by different oxygen concentrations. The results of the laboratory investigation of the potted plants suggested that the only site-specific (ecotypic) response of *M. congestus* was the light intensity at which the plants from the different sites were light saturated.

The light and temperature response of field plants under field conditions was not comparable to the light and temperature response of potted plants under laboratory conditions. This may have been due to the field results being obtained under differing water and soil nutrient regimes. The potted plants may also have had a reduced root mass compared to their field counterparts and the potted plants may have also have become root bound. Under field conditions the plants had differing light saturation points and optimal photosynthetic temperatures compared to the potted plants. This investigation thus did not support the hypothesis stated in this thesis. The data in this investigation thus may indicate that plants with as diverse habitats as *Mariscus congestus* that are removed from their natural habitats display rapid changes in gas exchange characteristics in response to their new microclimates, with few ecotypic physiological characteristics of the old habitat being retained.

CHAPTER 1

General Introduction, Aims and Objectives

INTRODUCTION

Mariscus congestus is a species with a wide range in South Africa (Gledhill 1981; Schönland 1922; Thiselton-Dyer 1900). Its range extends from Cape Town to Natal, and from the Transvaal to Kimberley. *M. congestus* also extends from the coast in the Eastern Cape, to the mountains near Graaff-Reinett and Murraysberg. In South Africa this species ranges from near sea level to above 2000m (Schönland 1922; Thiselton-Dyer 1900). *M. congestus* also occurs widely throughout Africa, except in the tropics (Hilliard 1987; Schönland 1922; Thiselton-Dyer 1900). Plants of *M. congestus* are also found in the Mediterranean region and in Australia (Schönland 1922; Thiselton-Dyer 1900).

Species with wide ranges usually undergo genotypic divergence in their habitats in response to their habitat, and the microclimates prevailing in that habitat (Osmond *et al.* 1980; Ledyard Stebbins 1963). The modification of the genotype to a habitat or niche result in a habitat type or ecotype of that species (Briggs & Walters 1986; Jones & Luchsinger 1986; Barbour *et al.* 1980; Osmond *et al.* 1980; Ledyard Stebbins 1963). An ecotype thus emphasises the genetic heterogeneity of a taxonomic species and the pervasive influence of the local environment at the morphological, physiological and successively more subtle levels of plant behaviour (Jones & Luchsinger 1986; Barbour *et al.* 1980; Osmond *et al.* 1980). An ecotype may or may not however, possess clear morphological differences, which enable them to be recognised in the field (Osmond *et al.* 1980; Ledyard Stebbins 1963). The physiological differences between ecotypes are however, correlated with the enzymatic and other biochemical adaptations that enable a plant to succeed in its habitat.eg. Sun ecotypes (plants in habitats with high light environments) will have higher light saturation points than plants from shade ecotypes (low light habitats) (Barbour *et al.* 1980). The ecotypic divergence of a specific plant (species) will eventually lead to that plant becoming genetically distinct from its original plant population. This will result in the separation of the plants at each different habitat into a subspecies or a variety of the original plant species (Jones & Luchsinger 1986).

Mariscus congestus thus seemed an excellent choice to determine if habitat conditions (i.e. aspect, temperature, soil moisture availability and light intensity) had resulted in this species becoming ecotypically distinct from other *M. congestus* populations. The hypothesis of this thesis was that **the removal of *Mariscus congestus* from three different natural environments (habitats) and their transferral to glasshouse conditions would not cause a shift in their gas exchange characteristics.** It was hoped that the results of this investigation would confirm the ecotype concept of Turesson, similar to the investigations of Milner and Hiesey (1964) on plants of *Mimulus* sp., Green (1969) and Slayter and Ferrar (1977) on plants of *Eucalyptus pauciflora* Sieb. ex Spreng..

The aim of this research was thus to investigate the gas exchange characteristics in the field and to compare the results obtained there, to the results obtained using potted material from the three different habitats (sites) in a new, controlled environment. In order to test the above hypothesis and to meet the aims of this research it was thus necessary to complete the following investigations. The leaf blade anatomy of *M. congestus* was investigated in some detail to ascertain the level of difference (if any) between the leaves of each site. The gas exchange characteristics were examined in the field over a period of a year to establish if any ecotypic characteristics were displayed in the field and to obtain light and temperature response data from each site. A comprehensive investigation of the gas exchange characteristics of potted plants from each habitat, placed in a new habitat (glasshouse) was also performed. The results of this investigation are presented and discussed in conjunction with the field and laboratory gas exchange investigation data.

CHAPTER 2

Materials and Methods

2.1 Plant site selection

A systematic survey of all rivers in the Albany and Bathurst districts was carried out to establish the distribution of *Mariscus congestus* (Vahl) C.B.Cl. in the Albany and Bathurst districts. Three sites were subjectively selected from the survey for the investigation (Fig. 1). The three sites were on the same geological strata (Cape Supergroup) and had the same annual rainfall area (approximately 500mm per annum). The sites were chosen on the basis of aspect (topographical), soil nutrient differences, soil form and difference in vegetational composition.

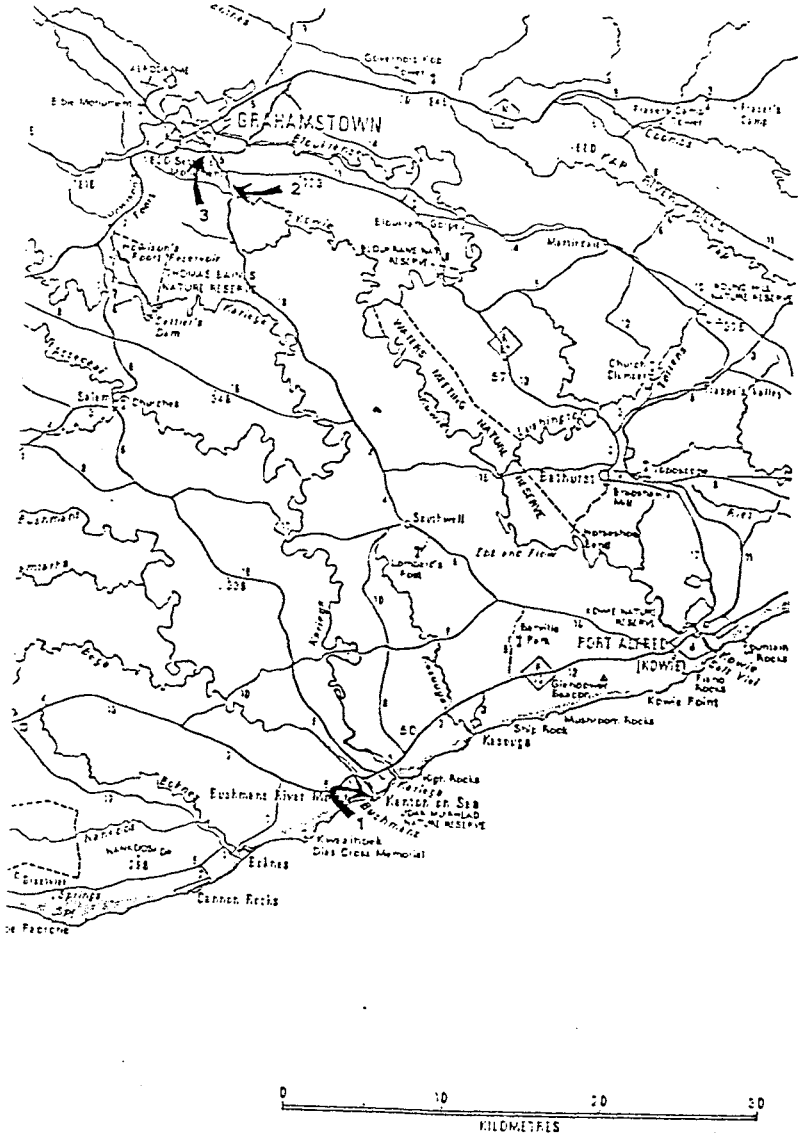

Fig. 1. Illustrates the location of study sites in the Albany and Bathurst districts. The arrows (⇐) show the location of the experimental sites 1, 2 and 3.

The three sites selected for the investigation were as follows:

Site 1) A sand dune blowout located between the coastal climax dune thicket on a Fernwood soil form (MacVicor *et al.* 1988) and a second serial colonized dune vegetation area, on a landward NW-facing dune, 150m from the Kariega river and 300m from the Bushmans river mouth, at Kenton-on-Sea in the Bathurst district of the Eastern Cape (Fig. 2).

Site 2) A seaward, SSE-facing slope approximately 250m from the crest of Woest hill, in a disturbed grassy fynbos habitat on a Katspruit soil form (MacVicor *et al.* 1988), 50m from the Southwell road, 1.0km from the Southwell turn-off, on the Port Alfred National road (Fig. 3).

Site 3) A landward, East-facing slope of Mountain Drive (Fig. 4), at the bottom of the farm Waterloo, 200m from the Grahamstown bypass on the Port Alfred National road, in a regenerating pasture habitat on the Villafontes soil form (MacVicor *et al.* 1988).

Fig. 2. Shows the sand dune blowout habitat (⇓) of site 1, located between the coastal climax dune thicket (A) and the second serial colonized sand dunes (B) at the Bushmans river mouth, in the Bathurst district of the Eastern Cape.

Fig. 3. Shows the disturbed grassy fynbos habitat of site 2 (⇓), near the crest of Woest Hill.

Fig. 4. Shows the shaded, disturbed pasture habitat of site 3 (⇓), located on the farm Waterloo near Grahamstown.

2.2 Leaf anatomy

The anatomy of the mature leaves from each site was investigated at the light microscope level. Small sections (approximately 2.0 x 0.5cm) of leaf tissue were excised from the mid-lamina regions of the mature leaves of *Mariscus congestus* from each site and fixed in formyl acetic alcohol (FAA). Leaf segments were then sequentially dehydrated in an ethanol and tertiary butyl alcohol series. The segments were infiltrated with Paraplast wax (Capital Enterprises, New Germany, South Africa), blocked and cut, using a Lietz Wetzlar Minot microtome (Wetzlar, West Germany) at a thickness of 7-10μm. Sections were mounted on clean slides with Haupts adhesive. The slides containing the mounted sections were dried at 45°C in a FSIE 2 Incubator (Labcon (PTY) Ltd., Krugersdorp, South Africa) and stained with safranine and Fast green CFC.

The sections of the leaves from each site were examined using a Zeiss Standard Junior 18 microscope (Carl Zeiss PTY. Ltd., Oberkochen, West Germany) and photographed using a Zeiss MC-63 camera and Agfa pan 25 ASA professional film.

2.3 Plant cultivation for laboratory investigations

Plants with intact root stocks were collected from each of the three study sites. Each plant was placed in a mixture of potting sand and beach soil (1:1 w/w). The potted plats were transfered to a glass-house and watered daily using rain water, in order to maintain the soil at field capacity during plant re-stabilization and regrowth. The plants were left in the glass-house until new growth had occured. Leaves were not used for the laboratory-based gas exchange experiments until they were of suitable maturity (approximately two weeks old).

During the colder months (June to September 1990), the glass-house was maintained above a minimum temperature of 15°C, using a thermostated 1800W Phillips blow heater (Phillips, Johannesburg, South Africa).

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2.4 Gas exchange

All gas exchange investigations were undertaken using a calibrated portable LCA-2 infra-red gas analyzer (IRGA), with an attached DL-2 data logger (The Analytical Development Co. Ltd., Hoddesdon, England). The IRGA was set up in open circuit, and carbon dioxide was measured in the differential mode.

Air was supplied from an outside source and pumped through self-indicating 8-mesh Drierite (Unilab, Krugersdorp, South Africa), into the reference port at a flow rate of 350ml.min^{-1} using an ADC-ASUM Mass Flow meter (Hoddesdon, England). Part of the external air supply was also passed through a Parkinson broad leaf cuvette (PLC-B) enclosing an attached leaf of known area. Air exiting from the leaf chamber was pumped to the analysis port of the IRGA. All gas exchange calculations were derived from the programme IRCAL 2.0 (Copyright Botha & Brown 1991), which utilizes the equations of Von Caemmerer and Farquhar (1981).

2.4.1 *Field gas exchange investigations*

Field gas exchange characteristics of mature leaves of *M. congestus* were monitored at each site once a month from April 1990 to March 1991. This entailed investigating the gas exchange response of the mid-laminar region of mature leaves, at 30min intervals from 8h30 to 17h00, on relatively cloudless days. A sun-facing leaf was used for all field gas exchanges investigations. Adjacent plants were not removed to facilitate maximum irradiance on the leaf surface of the experimental plants. All gas exchange experiments were conducted on leaves of similar age and the second leaf from the rhizome was used on the same plant. Due to the annual die-back of the plants at sites 2 and 3 over the one year

field study period, comparable plants that regrew in the same area as the plant investigated previously had to be used for the remaining gas exchange studies.

Readings were taken in the following manner. The cuvette was clamped onto the leaf and the humidity was left to stabilize (usually for 1 minute). Once the humidity within the cuvette had stabilized, three readings were taken at 30 second intervals, in order to minimize experimental error. Directly after the readings were taken the cuvette was removed and the LCA-2 IRGA switched off. The field set up for the IRGA is illustrated in Figs. 5-6. Graphs were plotted using Supercalc Ver. 5.0 (Computer Associates International Inc., San Jose, California, U.S.A.).

Figs. 5-6. Shows the IRGA set up at a field site (site 2). Fig. 5. Shows the gas analyser (A) and coupled ASUM mass flow meter (B). Fig. 6. Shows the PLC-B- broad leaf chamber (C) containing a leaf.

2.4.2 *Laboratory gas exchange investigations*

A portable LCA-2 IRGA was used for this investigation. The IRGA was set up in open circuit and differential mode (Fig. 7). The IRGA was pre-calibrated using standard gas mixtures containing known

CO_2 concentrations. The laboratory set-up was as follows. Outside air was pumped into a 25l buffer drum using an ADC WA 197B-9 exhaust pump (Hoddesdon, England). The air in the buffer system was used as the reference air source for the light and temperature experiments. In order to maintain the leaf in a stream of high relative humidity between the experimental readings, the air stream to the PLC-B leaf chamber was diverted to a cuvette containing a wet filter paper surface, which ensured a high relative humidity (RH between 50 and 65%) in the leaf chamber, to prevent stomatal closure or leaf stress. Immediately prior to taking readings, the air flow was redirected to a dry air bypass. Once the relative humidity had stabilized, readings were taken at 30 second intervals at that light intensity, temperature or CO_2 concentration. Unless otherwise stated each experiment was repeated three times on different leaves of approximately the same morphological age as the leaves used in the field investigations. Thus at least nine data points were taken at each light intensity, temperature or CO_2 concentration. The temperature and light intensities were varied using the procedures discussed below.

2.4.3 *Light and temperature response*

2.4.3.1 *Temperature response*

Laboratory-based photosynthetic gas exchange of the mid-laminar regions of attached mature leaves for the plants from each site was investigated under temperature-controlled conditions. A heat-sink, was attached to the undersurface of the Parkinson broad leaf chamber and thermoregulated using a Lauda RMS-6 recirculation chiller (Optolabor, Johannesburg, South Africa). Incident light levels were varied during the constant temperature experiments. Light response experiments were carried out at the following temperatures: 20, 25, 30, 35 and 40°C, to determine the plants temperature responses. The resultant temperature response curves were plotted using Fig-P version 5.0b (Biosoft, Milltown, U.S.A.), on a 8086 or 80286 based computer.

2.4.3.2 *Light response*

Actinic light was provided by using a Vialox NAV-T400W high pressure sodium lamp (Osram, Johannesburg, South Africa). Varying light intensity was achieved by placing shade cloth netting of differing thicknesses between the leaf surface and the lamp. The light intensities at which CO_2 assimilation rates were monitored were as follows: 0, 12.5, 25, 50, 75, 125, 250, 500, 750, 1000, 1500, 2000 and 2400µmol $m^{-2}.s^{-1}$. Temperatures were carefully monitored and maintained within 0.5°C of the desired experimental temperature.

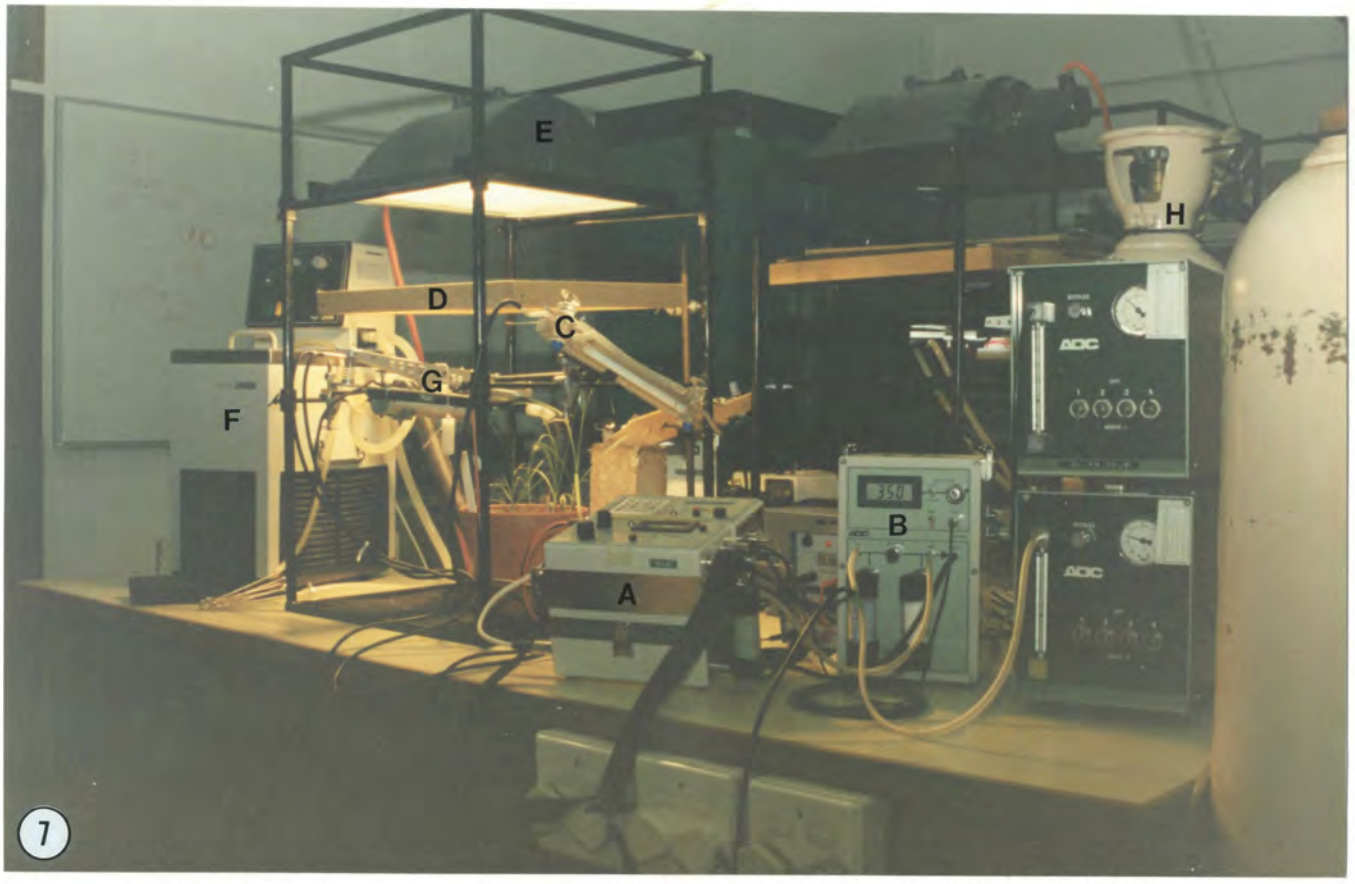


Fig. 7. Shows the laboratory infra-red gas analyser (IRGA) set-up. A- The ADC LCA-2 IRGA with attached DL-2 data logger; B- an ADC-ASUM mass flow meter; C- humid/dry air bypass system; D- shade cloth; E Vialox NAV-T400W high pressure sodium lamp; F- RMS-6 Lauda & G- Parkinson broad leaf cuvette (PLC-B) with attached heat sink. Analytical gas bottles can be seen in the background.

2.4.4 ***The effect of varying carbon dioxide and oxygen concentration on photosynthesis***

Certified gases (Fedgas, Port Elizabeth, South Africa) were used for all experiments involving CO_2 or O_2 concentration manipulation. Potted plants from each site were subjected to different oxygen

concentrations to ascertain if inherent similarities or differences in photosynthetic response could be measured experimentally in the plants under laboratory conditions. Attached leaves were clamped into the broad leaf cuvette at their optimal photosynthetic temperature and at a light intensity of 1500µmol $m^{-2}.s^{-1}$. After the leaf had been maintained under optimal conditions for 30min, the response to different concentrations of carbon dioxide was determined. CO_2 was stripped from 600µl.l^{-1}, at 50µl.l^{-1} steps, between 600 and 0µl.l^{-1} using an ADC GD-600 gas diluter (Hoddesdon, England). The oxygen concentration was monitored using a Beckman OM-14 oxygen analyser (Beckman, Johannesburg, South Africa) during each CO_2-stripping experiment. The oxygen concentration was monitored to ensure that the concentration did not vary during the experiment. Each experiment was repeated using the following oxygen concentrations: 0, 8, 16, and 21%. Each experiment was replicated four times at each oxygen concentration and three readings were taken at each CO_2 concentration. Curves of CO_2 assimilation vs internal CO_2 concentration (A/Ci) were plotted using Fig-P. Graphs represent the means of all data sets for the experiments.

2.4.5 **Graphical manipulation of laboratory gas exchange data**

2.4.5.1 ***Light and CO_2 concentration data***

Graphs of the data for the light and CO_2 concentration were constructed using FIG-P, using the monomolecular formula described in Causton & Dale (1990). Causton and Dale's (1990) monomolecular formula was used to extrapolate the actual experimental data points. The Ci concentrations were extrapolated at 50µl.l^{-1} intervals between 0 and 600µl.l^{-1} from the raw data curves.

Thus the light intensity and CO_2 concentration for each experiment was extrapolated from the raw data curves, and CO_2 assimilation rates at light intensities of: 0, 12.5, 25, 50, 75, 125, 250, 500, 750, 1000, 1500, 2000 and 2400µmol $m^{-2}.s^{-1}$ were determined.

2.4.5.2 *Kinetic analysis of apparent V_{max} and apparent Km values of CO_2 assimilation in attached leaves*

Reciprocal plots (1/A vs 1/Ci-τ) were plotted using the methods detailed in Ku and Edwards (1977). The plots were used to extrapolate the $1/^{app}Km$ and $1/^{app}V_{max}$ intercepts. Where the $^{app}V_{max}$ is the apparent value of maximum assimilation rate, under a particular set of conditions achieved at substrate saturation, and the ^{app}Km is the apparent Michaelis-Menton constant of the carboxylation process by the leaf and reflects the stability of enzyme-substrate interaction of the photosynthetic process (Stryer 1981; Clark & Switzer 1977).

2.6 Field environmental parameters recorded

The field environmental parameters recorded on the same days as the monthly field gas exchange investigations were, mean daily temperature, light intensity and soil moisture content.

2.6.1 *Temperature and light intensity*

Light intensity and temperature readings were recorded simultaneously with the gas exchange data by sensors located within the broad leaf chamber. This data was used to calculate the mean daily light intensity and temperature for each site using Supercalc Ver. 5.0. Graphs were potted using Fig-p and Supercalc Ver. 5.0.

2.6.2 *Soil moisture content*

A 100g sample of the soil from each site was taken at a depth of 10cm, as this was found to be the mean rooting depth of the plants at each site. Each sample was removed at 12h00, and at the same time as the gas exchange characteristics were being recorded at that site. Soil moisture content was calculated from the soil sample using three 25g replicates. The sample was taken from the area surrounding the plants at that site. Each twenty five gram soil sample from the field (wet wt) was weighed and oven

dried in a FSIE 2 incubator at 95°C for 24h and reweighed to obtain the dry weight. The percentage moisture of the soil was then calculated using the following equation.

$$\% \text{ Moisture} = \frac{(\text{wet wt} - \text{dry wt})}{(\text{wet wt})} * 100$$

Graphs were plotted using Supercalc Ver. 5.0. The above mentioned method of analysing soil samples are those of (Loock *et al.* 1990).

2.7 Statistical tests

The statistical tests used for comparing the data in the field and the laboratory were the Students t-test for two sample means and Chi-square contingency table testing at a 99% level of significance.

CHAPTER 3

Anatomy of *Mariscus congestus*

leaves from sites 1, 2 and 3

3.1 INTRODUCTION

The Leaf anatomy of the family Cyperaceae is unique in that there are many differences in vascular bundle sheath anatomy when compared to other C_4 monocotyledonous plants (Carolin *et al.* 1977; Brown 1975). The leaves of C_4 Cyperaceae can be divided into three groups based on the arrangement of the radiating (Kranz) mesophyll, mestome sheath and primary carbon reducing (PCR) or Kranz bundle sheath cells (Ueno *et al.* 1986; Laetsch 1974). The vascular bundle sheaths tend to be cylindrical, with no air spaces between them or their component cells (Laetsch 1974). The three groups are known as the Chlorocyperiod, Fymbristylloid and Rhynchosporoid group, respectively. They are distinguished on the basis of the arrangement of, and number of sheaths that surround the leaf vascular bundles.

The Fymbristylloid group is characterised by the presence of three sheaths surrounding the vascular bundle (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980; Carolin *et al.* 1977; Johnson & Brown 1973). The outer sheath is parenchymatous and is encircled by a layer of radiating mesophyll. The middle sheath is described as a mestome sheath and the innermost sheath is described as the Kranz sheath, which in the larger vascular bundles, is interrupted by two large metaxylem vessels (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980; Carolin *et al.* 1977). The contents of the Kranz sheath are similar to those found in NADP-ME grasses, in that the plastids have underdeveloped grana and are arranged centrifugally (Carolin *et al.* 1977).

In the Rhynchosporoid group the vascular bundles are surrounded by two vascular bundle sheaths and an outer radiating mesophyll (Takeda *et al.* 1985, 1980). The outer sheath or parenchymatous sheath is however, often hard to distinguish from the radiating mesophyll and is often incomplete (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980). The parenchymatous sheath is usually incomplete on the ad- and abaxial poles of the vascular bundle (Takeda *et al.* 1985, 1980). The radiating mesophyll at these poles of the vascular bundle is thus often in direct contact with the inner bundle sheath (Takeda *et al.* 1980). The inner sheath is described as the Kranz sheath, which in larger bundles, is not interrupted by large metaxylem vessels (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980).

The Chlorocyperiod group species are characterised by the presence of two vascular bundle sheaths arranged around the vascular bundles (Takeda *et al.* 1985; Brown 1975; Laetsch 1974; Johnson & Brown 1973). The outermost sheath is termed the mestome sheath. The mestome sheath is in direct contact and encircled by the radiating mesophyll (Carolin *et al.* 1977; Brown 1975). The mestome sheath is also in direct contact with two large metaxylem vessels in the large vascular bundles (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980; Hesla *et al.* 1982; Brown 1975; Laetsch 1974). Mestome sheath cells are small and possess no chloroplasts (Laetsch 1974). It has been suggested by Johnson and Brown (1973) that the mestome sheath may be recognised as the equivalent to a starch sheath or endodermis, since it resembles the endodermis of roots and stems. As well as the aforementioned similarities to the endodermis of roots and stems the mestome sheath also possess a suberin lamellae and thick walls (Brown 1975; Laetsch 1974). The innermost sheath is described as the Kranz sheath, which is entire in the smaller vascular bundles. In larger bundles, the sheath is often interrupted by two large metaxylem vessels, whilst in other vascular bundles, the sheath is restricted to the phloem side (Ueno *et al.* 1986; Hesla *et al.* 1982; Carolin *et al.* 1977; Brown 1975).

3.1.1 C_4 subtypes in the family Cyperaceae

The presence of the three C_4 subtypes within the Fymbristylloid, Rhynchosporoid and Chlorocyperiod groups is still a point of contention, with some authors maintaining that all Cyperaceae are C_4, NADP-ME (Carolin *et al.* 1977; Brown 1975). This theory however, appears suspect in the light of the discovery of the NAD-ME subtype in plants of *Eleocharis* sp., by Bruhl *et al.* (1987) and the possibility that *Cyperus fastigiatus* is either NAD-ME or PCK (Sonnenberg 1989).

3.1.2 Characteristics for genus level identification

Not only is the aforementioned vascular bundle sheath arrangement unique to the Cyperaceae but, other anatomical characteristics have proved to be valuable in the identification in this family to genus level (Metcalfe 1969). In all there are seven anatomical characters that are important and useful diagnostically. These characters are as follows.

1) The relative thickness of the adaxial epidermis, as compared with the abaxial epidermis.

2) The presence or absence of a hypodermis beneath either or both the leaf ad- and abaxial epidermis.

3) The difference in the distribution patterns of sclerenchyma and its relation to the vascular bundles, in the form of girders, each connecting the epidermis to the bundle sheath of a sub-adjacent vascular bundles, or strands, which usually abut the epidermal cells, but have no direct contact with vascular bundles. On occasion sclerenchyma may abut the vascular bundles, and extend towards the epidermis, but does not reach the epidermis, forming a partial girder. Sclerenchyma cells may also form caps that are attached to a vascular bundle.

4) The presence or absence of, different sizes and numbers of intercellular air cavities embedded in the chlorenchyma between the vascular bundles. These are formed by the breakdown of translucent cells, which may be either lobed or stellate.

5) The presence or absence of buliform cells. These bands of cells are usually found in the adaxial epidermis, and usually lie above the midrib.

6) The arrangement of the vascular bundles. In a transverse section of a dorsiventral leaf, vascular bundles usually form a single row, composed of large, intermediate and small bundles. In *Mariscus*, vascular bundles may occur in two horizontal rows. Air cavities are frequently encircled by small vascular bundles. The special arrangements of the vascular bundles are also characteristic of isobilateral, pseudodorsiventral and cylindrical leaves.

7) Distinctions between the C_4 groups based on the number and arrangement of sheaths surrounding the vascular bundles (described above) (Metcalfe 1969).

3.2 RESULTS

3.2.1.1 General anatomical description of the leaf material from site 1

The leaves of plants from this site have a thick adaxial and a thin abaxial cuticle (Figs. 8-11). The abaxial epidermis is composed of small cells, which are rounded in shape. The adaxial epidermis is

composed of large, square-shaped cells in the lamina region, with buliform cells in the midrib area. A clear non-lignified hypodermis (1 to 2 cells in thickness) occurs beneath the adaxial epidermis and above the abaxial epidermis (1 cell thick). There are numerous air cavities in the lamina of the leaf, and are located between the regions of stellate parenchyma.

Stellate parenchyma occurs between the abaxial vascular bundles. Little mesophyll tissue occurs between the radiating mesophyll, the stellate parenchyma and the hypodermis. Numerous tannin-containing cells are distributed throughout the lamina, and are usually associated with the ad- and abaxial hypodermis. Stomata are found exclusively in the abaxial epidermis. The leaves from site 1, are thus hypostomatous.

The large vascular bundles are associated with adaxial hypodermal sclerenchymatous strands. The epidermal cells above these hypodermal sclerenchymatous strands are all smaller than the adjacent adaxial epidermal cells. The abaxial hypodermal sclerenchymatous strands are all located directly beneath the large, intermediate and small abaxial vascular bundles, as well as between the regions of stellate parenchyma. The midrib bundle however, has two hypodermal sclerenchymatous strands associated with it.

The vascular bundles in the lamina are arranged in three distinct rows. Large bundles are associated with a few small vascular bundles in the adaxial row. Intermediate vascular bundles occupy the central row. And a few small vascular bundles occupy the abaxial row. The arrangement of vascular bundles between large bundles is illustrated in Fig. 8. The maximal lateral cell count between vascular bundles is less than four cells, indicating C_4 anatomy.

Radiating mesophyll is associated with all vascular bundles (Figs. 8, 10-11). All vascular bundles are surrounded by two bundle sheaths. The outer (mestome sheath), is located directly beneath the radiating mesophyll and is composed of compact, thick walled cells. Lignified mestome sheath cells abut the

large metaxylem vessels in the midrib and large vascular bundles. The inner bundle sheath termed the Kranz bundle sheath, is located inside the mestome sheath, and is directly adjacent to the vascular tissues. Kranz sheath cells contain large centrifugally arranged chloroplasts. The Kranz sheath in the midrib, large and intermediate vascular bundles are interrupted by two large metaxylem vessels.

3.2.1.2 **Brief description of the vascular bundles of site 1 leaves**

3.2.1.2.1 *Midrib*

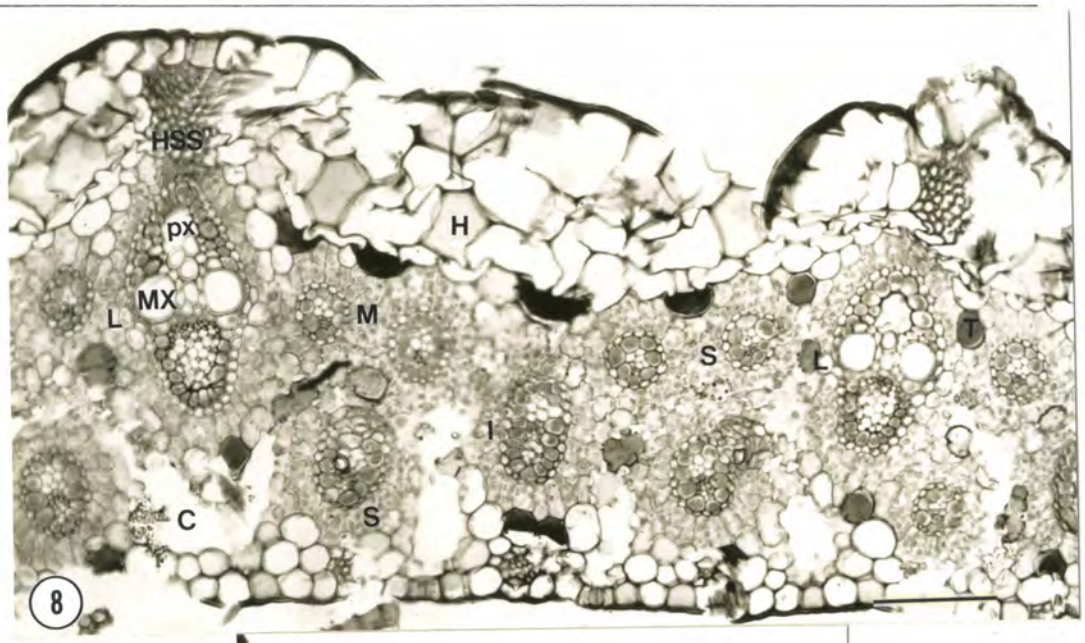
The midrib bundle is associated with a large adaxial sclerenchymatous cap, which interrupts the radiating mesophyll and the hypodermis adaxially (Fig. 9). The midrib contains four large metaxylem vessels (MX) and associated protoxylem lacunae (px), two of the metaxylem vessels interrupt the Kranz sheath. The large metaxylem vessels are bridged by tracheary elements. The phloem consists of vascular parenchyma, companion cells and metaphloem sieve tubes.

3.2.1.2.2 *Large vascular bundles*

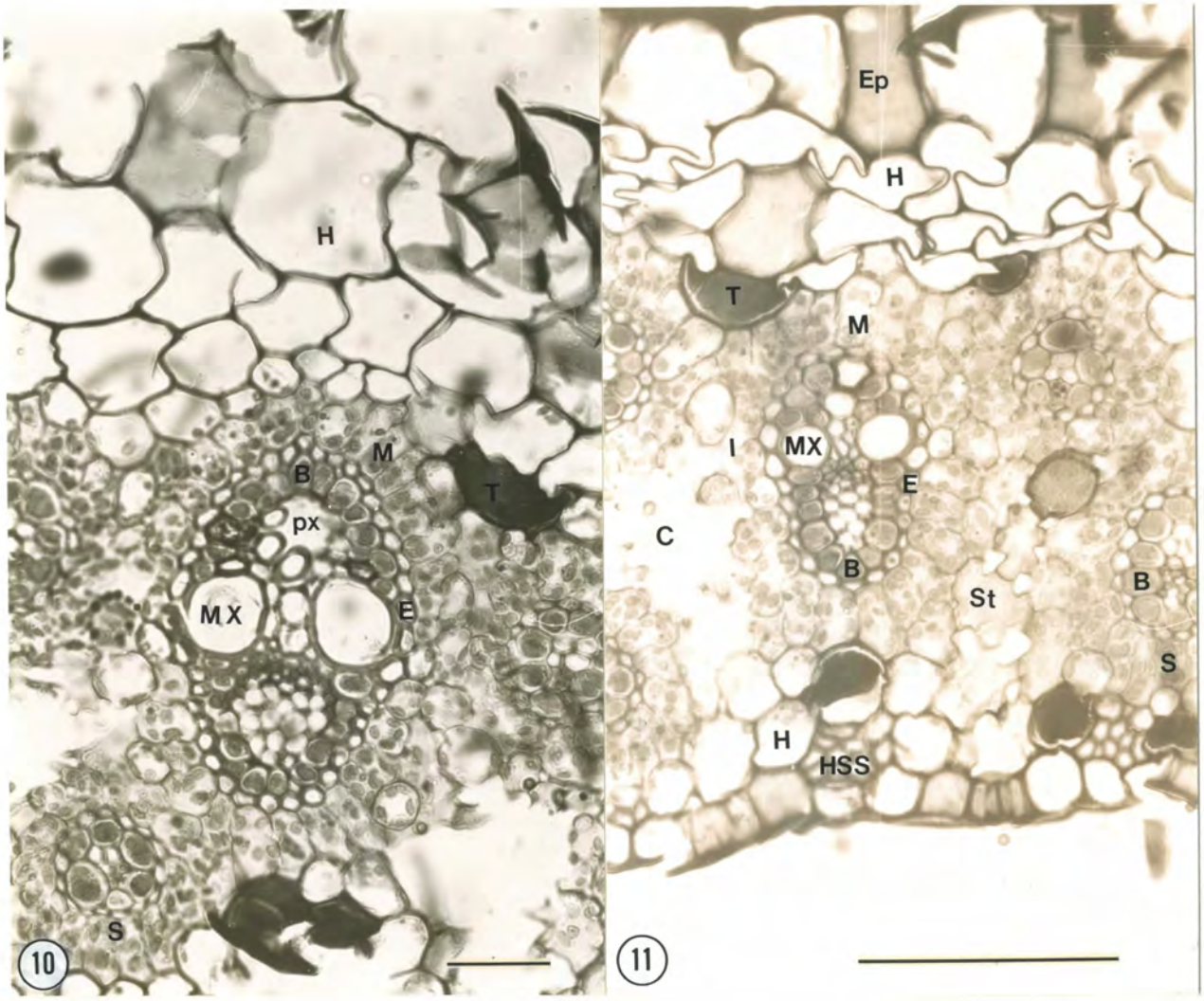
The radiating mesophyll is entire and not interrupted by a sclerenchymatous cap (Fig. 10). A large adaxial protoxylem lacunae (px) is also evident. The large bundles often contain intact protoxylem. Only two large metaxylem vessels are present. Both these vessels interrupt the Kranz bundle sheath laterally. A tracheid bridge connects the two large metaxylem vessels. The phloem consists of vascular parenchyma, companion cells and metaphloem sieve tubes.

3.2.1.2.3 *Intermediate vascular bundles*

The radiating mesophyll is entire and not interrupted by a sclerenchymatous girder, strand or cap (Fig. 11). The Kranz sheath is interrupted by two large metaxylem vessels and one small protoxylem vessel. The large metaxylem vessels are bridged by tracheary elements. The phloem consists of vascular parenchyma, companion cells and metaphloem sieve tubes.



Figs. 8 & 9. Show the leaf anatomy of the mature leaves of site 1 plants. Fig. 8. Shows the lamina arrangement of vascular bundles from one large bundle to the next. Fig. 9. Shows the midrib vascular bundle and surrounding tissue. (B)= Kranz bundle sheath; (C)= cavity once filled with stellate parenchyma; (E)= mestome sheath; (H)= hypodermis; (HSS)= hypodermal sclerenchymatous strands; (I)= intermediate vascular bundle; (L)= large vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (px)= protoxylem lacunae; (S)= small vascular bundle; (Sc)= sclerenchymatous cap and (T)= tannin cell. The bar = 20μm.



Figs. 10 & 11. Show the large, intermediate and small vascular bundles and surrounding tissue of site 1 mature leaves. Fig. 10. Shows a large vascular bundle and surrounding tissues. Fig. 11. Shows the intermediate, small vascular bundles and surrounding leaf tissues. (B)= Kranz bundle sheath; (C)= cavity once filled with stellate parenchyma; (E)= mestome sheath; (Ep)= adaxial epidermis; (H)= hypodermis; (HSS)= hypodermal sclerenchymatous strands; (I)= intermediate vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessels; (S)= small vascular bundle; (St)= stellate parenchyma and (T)= tannin cell. The bar = 20µm.

3.2.1.2.4 *Small vascular bundles*

The radiating mesophyll in the small bundles is entire and not interrupted by a sclerenchymatous girder, strand or cap (Fig. 10 & 11). The xylem is composed of small metaxylem vessels only. Tracheids do not bridge the metaxylem vessels. Little phloem (5 to 6 cells) is present and consists of vascular parenchyma, companion cells and metaphloem sieve tubes.

3.2.2.1 **General anatomical description of the leaf material from site 2**

Transections of leaves from site 2 revealed that the adaxial cuticle is far thicker than the abaxial cuticle (Fig. 12). The adaxial epidermis is composed of large, rectangular cells in the lamina and buliform cells in the midrib region. The abaxial epidermis is composed of compact, rounded cells. Numerous tannin-containing cells are found in the lamina and midrib regions, in close spatial association with the vascular bundles, adjacent both the ad- and abaxial epidermis. Stomata are found in the abaxial epidermis, below the large regions of stellate parenchyma.

Alternating large bundles are associated with adaxial hypodermal sclerenchymatous strands. The epidermal cells above these strands are composed of compact cells, which are smaller than the surrounding epidermal cells. In contrast, the abaxial hypodermal sclerenchymatous strands are all adjacent the abaxial row of large and intermediate bundles. The abaxial hypodermal sclerenchymatous strands are separated from each other by large regions of stellate parenchyma. Two abaxial hypodermal sclerenchymatous strands are also associated with the midrib.

Unlike site 1 plants, the vascular bundles within the leaves of site 2 plants are arranged in two rows in the lamina, and in one row in the midrib region. The adaxial bundles consists of small vascular bundles only. The lower or abaxial row, consists of alternating large and intermediate vascular bundles. The arrangement of vascular bundles is evident in Fig. 12. The regions between the large and intermediate bundles is occupied by stellate parenchyma. The maximal lateral cell count between vascular bundles is less than four cells, again indicative of C_4.

Similar to site 1 leaves, the vascular bundles of site 2 leaves are surrounded by two vascular bundle sheaths (Figs. 12-15). The outer or mestome sheath, is located beneath the radiating mesophyll, and is composed of small, thick-walled cells, which contain no visible cellular contents. The mestome sheath cells are lignified adjacent the two large metaxylem vessels in the midrib and large vascular bundles. The inner or Kranz sheath, is composed of large thin-walled cells, which contain large centrifugally-arranged chloroplasts. The Kranz sheath is interrupted by two large metaxylem vessels in the midrib and large bundles. The phloem tissue in all bundles, is comprised of vascular parenchyma cells, companion cells and metaphloem sieve tubes.

3.2.2.2 **Brief description of the vascular bundles of site 2 leaves**

3.2.2.2.1 *Midrib*

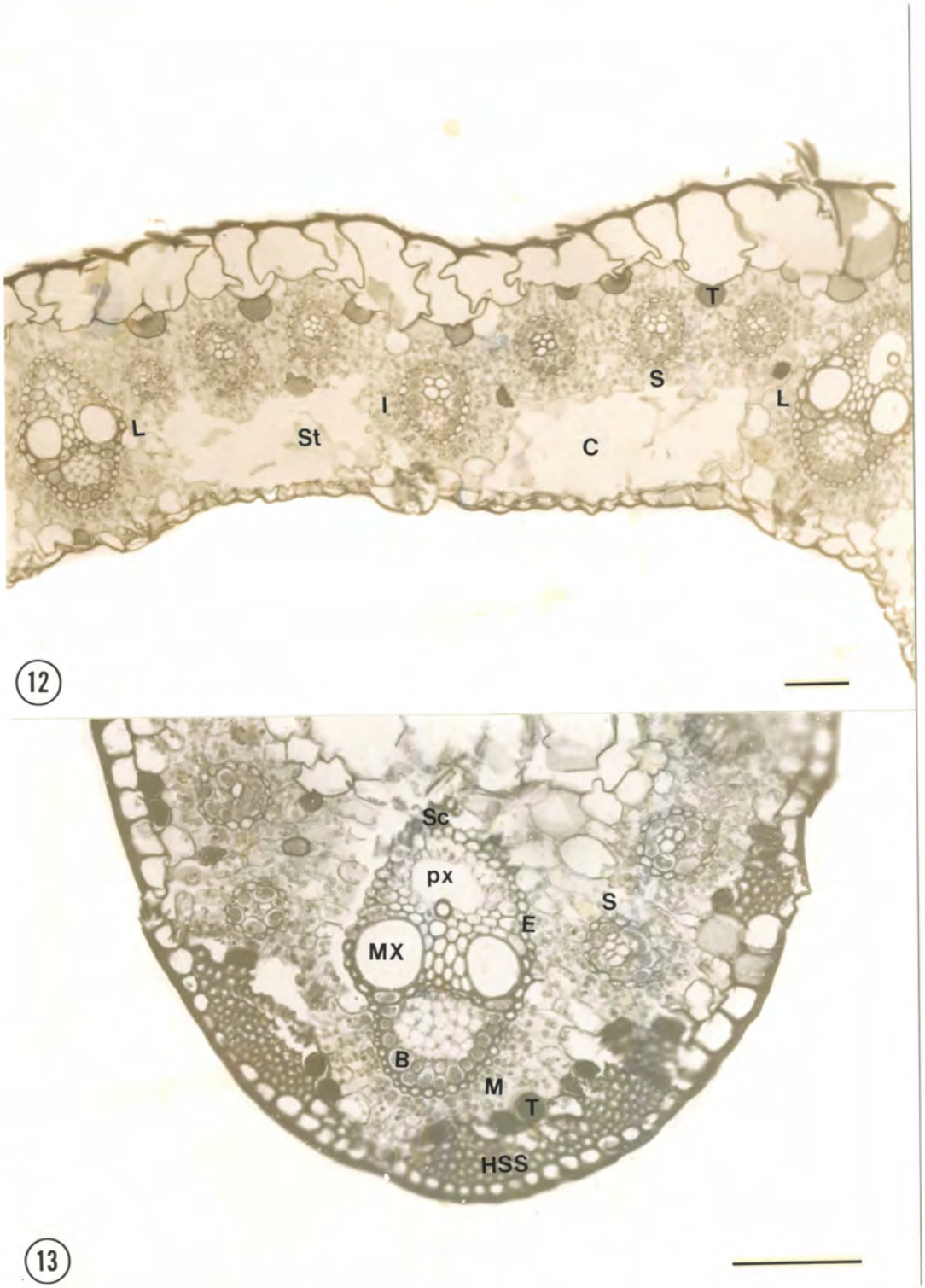
A large adaxial sclerenchymatous cap is situated on the adaxial pole of the bundle (Fig. 13). The radiating mesophyll is restricted to the phloem side of the midrib bundle. The midrib contains two large metaxylem vessels (MX) and an associated protoxylem lacunae. The two large metaxylem vessels interrupt the Kranz sheath and are bridged by tracheary elements.

3.2.2.2.2 *Large vascular bundles*

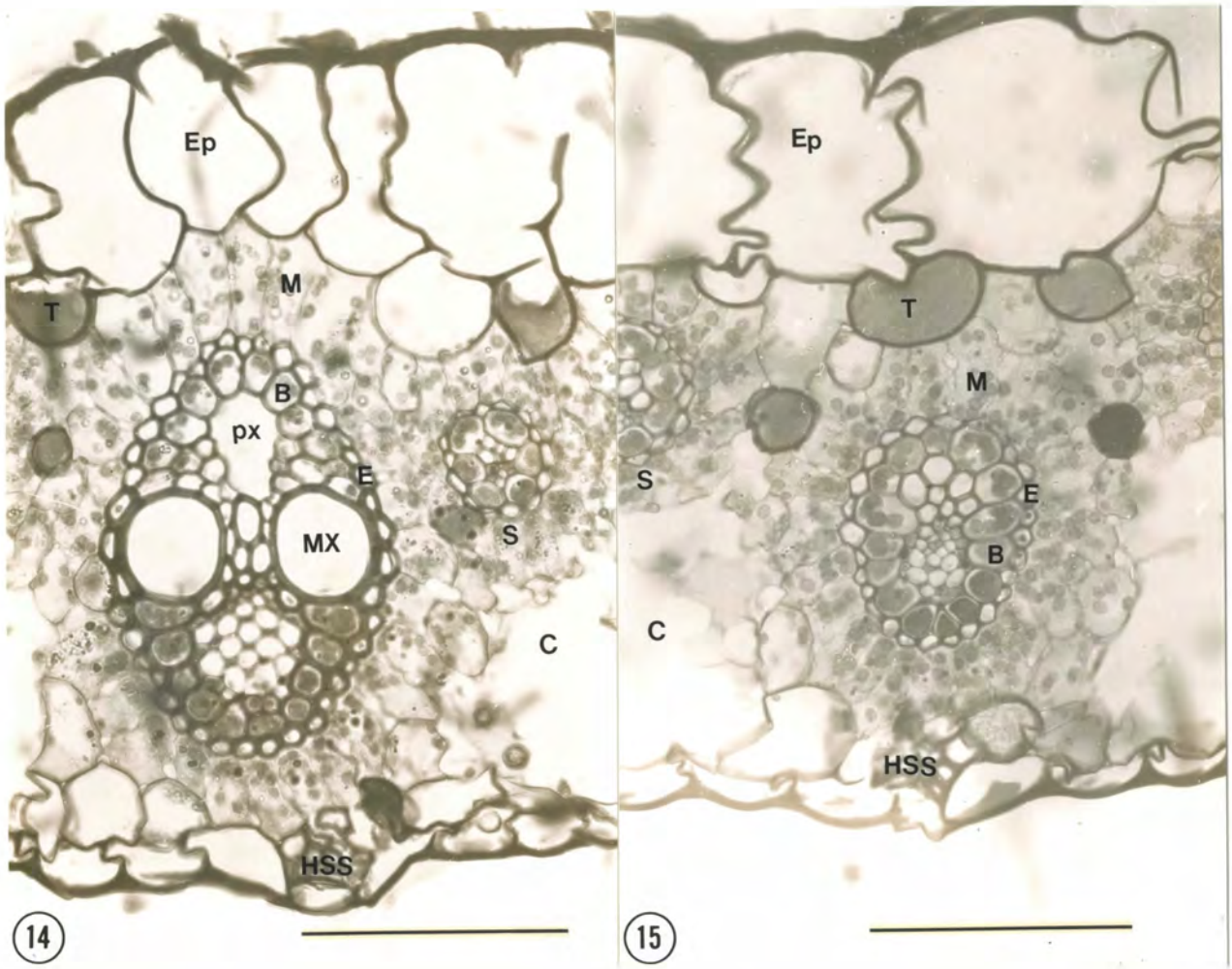
Sclerenchymatous caps are absent from the adaxial pole of the large bundles and the radiating mesophyll is entire and is not restricted to the phloem half of the vascular bundle. The xylem consists of two large metaxylem vessels and associated tracheary elements. A large protoxylem lacunae is evident in these bundles.

3.2.2.2.3 *Intermediate vascular bundles*

The radiating mesophyll is entire and not restricted to the phloem half of the vascular bundle (Fig. 15). The Kranz sheath is not interrupted by two large metaxylem vessels and there are no protoxylem lacunae. The xylem is composed of metaxylem vessels only.



Figs. 12 & 13. Shows anatomy of the mid-lamina and midrib regions of mature site 2 leaves. Fig. 12. Shows the lamina vascular bundle arrangement and Fig. 13, shows the midrib region. (B)= Kranz bundle sheath; (C)= cavity once filled with stellate parenchyma; (E)= mestome sheath; (HSS)= hypodermal sclerenchymatous strand; (I)= intermediate vascular bundle; (L)= large vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (px)= protoxylem lacunae; (S)= small vascular bundle; (Sc)= sclerenchymatous cap; (St)= stellate parenchyma and (T)= tannin cell. The bar = 20μm.



Figs. 14 & 15. Show large, intermediate and small vascular bundles in the mid-laminar region of the mature leaves from site 2. Fig. 14. Shows the large vascular bundle and surrounding tissues. Fig. 15. Shows an intermediate and small vascular bundle, and surrounding tissues. (B)= Kranz bundle sheath; (C)= cavity once filled with stellate parenchyma; (E)= mestome sheath; (Ep)= epidermal cell; (HSS)= hypodermal sclerenchymatous strand; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (px)= protoxylem lacunae; (S)= small vascular bundle and (T)= tannin cell. The bar = 20μm.

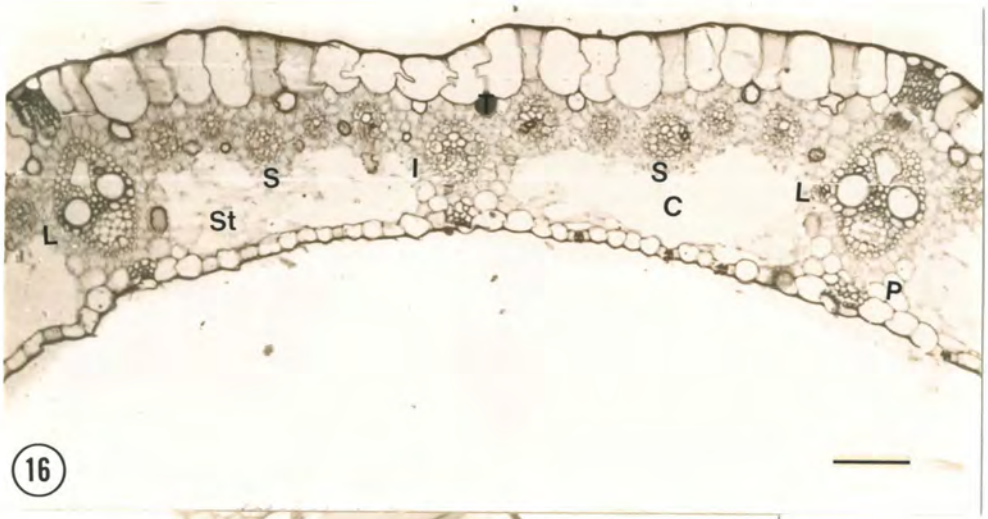
3.2.2.2.4 *Small vascular bundles*

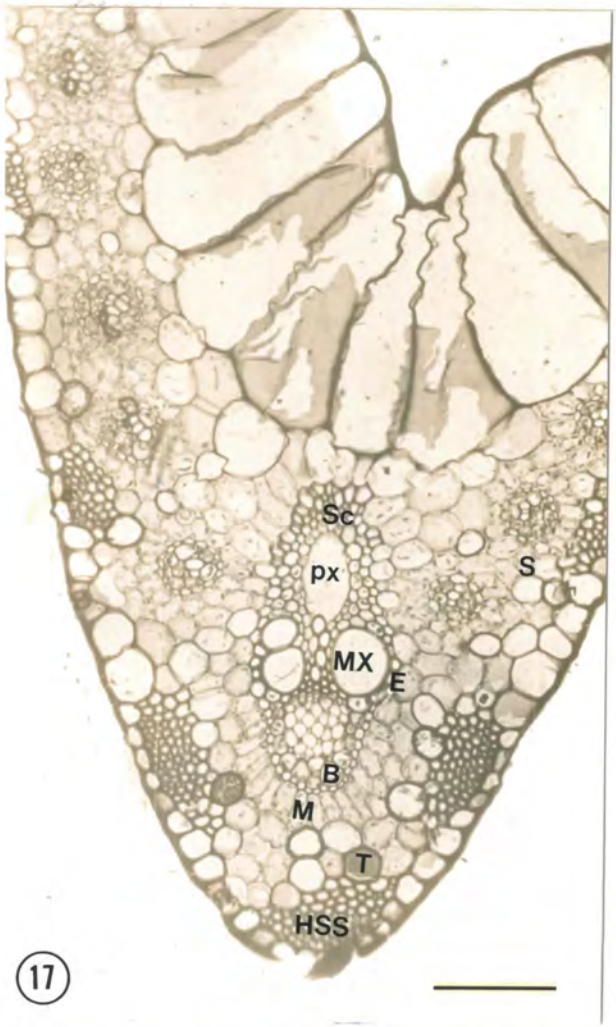
The radiating mesophyll in the small bundles is entire and not restricted to the phloem half of the vascular bundle (Fig. 14 & 15). The xylem is composed of a few small metaxylem vessels. No tracheary elements bridge the metaxylem vessels.

3.2.3.1 **General anatomical description of the leaf material from site 3**

The leaf anatomy of site 3 plants is very similar to the leaves of site 2 plants. The leaves of site 3 have thick ad- and thin abaxial cuticles, similar to the leaves of both sites 1 and 2. The adaxial epidermis is composed of large, oval shaped cells in the lamina and buliform cells in the midrib region. The abaxial epidermis is composed of compact rounded cells. Parenchymatous bridges link the large and intermediate vascular bundles to the abaxial epidermis (Fig. 16). The tannin-containing cells are associated with the vascular bundles (Figs. 16-17). Most of these tannin-containing cells are located directly beneath the adaxial epidermis between the vascular bundles. The stomata are located in the abaxial epidermis, below the stellate parenchyma regions (Fig. 16).

Each large bundle is associated with an adaxial hypodermal sclerenchymatous strand (Fig. 16). Ad- and abaxial epidermal cells associated with the hypodermal sclerenchymatous strands are more compact than the adjacent epidermal cells. Large and intermediate bundles have an associated abaxial hypodermal sclerenchymatous strand. The midrib has three hypodermal sclerenchymatous strands associated with it (Fig. 17).





Figs. 16 & 17. Show the lamina and midrib regions of the mature leaves from site 3. Fig. 16. Shows the lamina arrangement of vascular bundles and Fig. 17, shows the midrib region. (B)= Kranz bundle sheath; (C)= air cavity once filled with stellate parenchyma; (E)= mestome sheath; (HSS)= hypodermal sclerenchymatous strand; (I)= intermediate vascular bundle; (L)= large vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (P)= parenchymatous bridge; (px)= protoxylem lacunae; (S)= small vascular bundle; (Sc)= sclerenchymatous cap; (St)= stellate parenchyma and (T)= tannin cell. The bar = 20μm.

There are two rows of vascular bundles in the lamina (Fig. 16), an ad- and an abaxial row, similar to site 2. In the midrib region however, there is only one row of vascular bundles (Fig. 17). The adaxial row in the lamina is comprised only of small bundles. The abaxial row, is however, composed of large and intermediate bundles. The arrangement between the large bundles is illustrated in Fig. 16. Between the alternating, abaxial large and intermediate bundles are large regions of stellate parenchyma. The maximal lateral cell count between vascular bundles is less than four cells, which is indicative of C_4 photosynthetic pathway.

Radiating mesophyll is associated with all the vascular bundles (Figs. 16, 17, 19-21). All the vascular bundles also have two associated vascular bundle sheaths. The outer sheath or mestome sheath is located directly inside the radiating mesophyll. The mestome sheath is composed of compact, thick walled cells, which are lignified adjacent three large metaxylem vessels in the midrib. The mestome sheath in the large bundles is also lignified adjacent two large metaxylem vessels. The inner sheath or Kranz bundle sheath is composed of thin walled cells with small chloroplasts. The arrangement of chloroplasts within the Kranz bundle sheath is centrifugal. The Kranz bundle sheath is also interrupted by three large metaxylem vessels in the midrib and by two large metaxylem vessels in the large bundles. The phloem tissue in all bundles, is comprised of vascular parenchyma cells, companion cells and metaphloem sieve tubes.

3.2.3.2 **Brief description of the vascular bundles of site 3 leaves**

3.2.3.2.1 *Midrib*

A large adaxial sclerenchymatous cap is situated on the adaxial pole of this bundle (Figs. 17-18). The radiating mesophyll is restricted to the phloem half of the midrib bundle. The midrib contains three large metaxylem vessels (MX) and associated protoxylem lacunae (px), the three large metaxylem vessels also interrupt the Kranz bundle sheath. The Kranz bundle sheath similar to the radiating

mesophyll is restricted to the phloem half of the vascular bundle. The large metaxylem vessels are bridged by tracheary elements.

3.2.3.2.2 *Large vascular bundles*

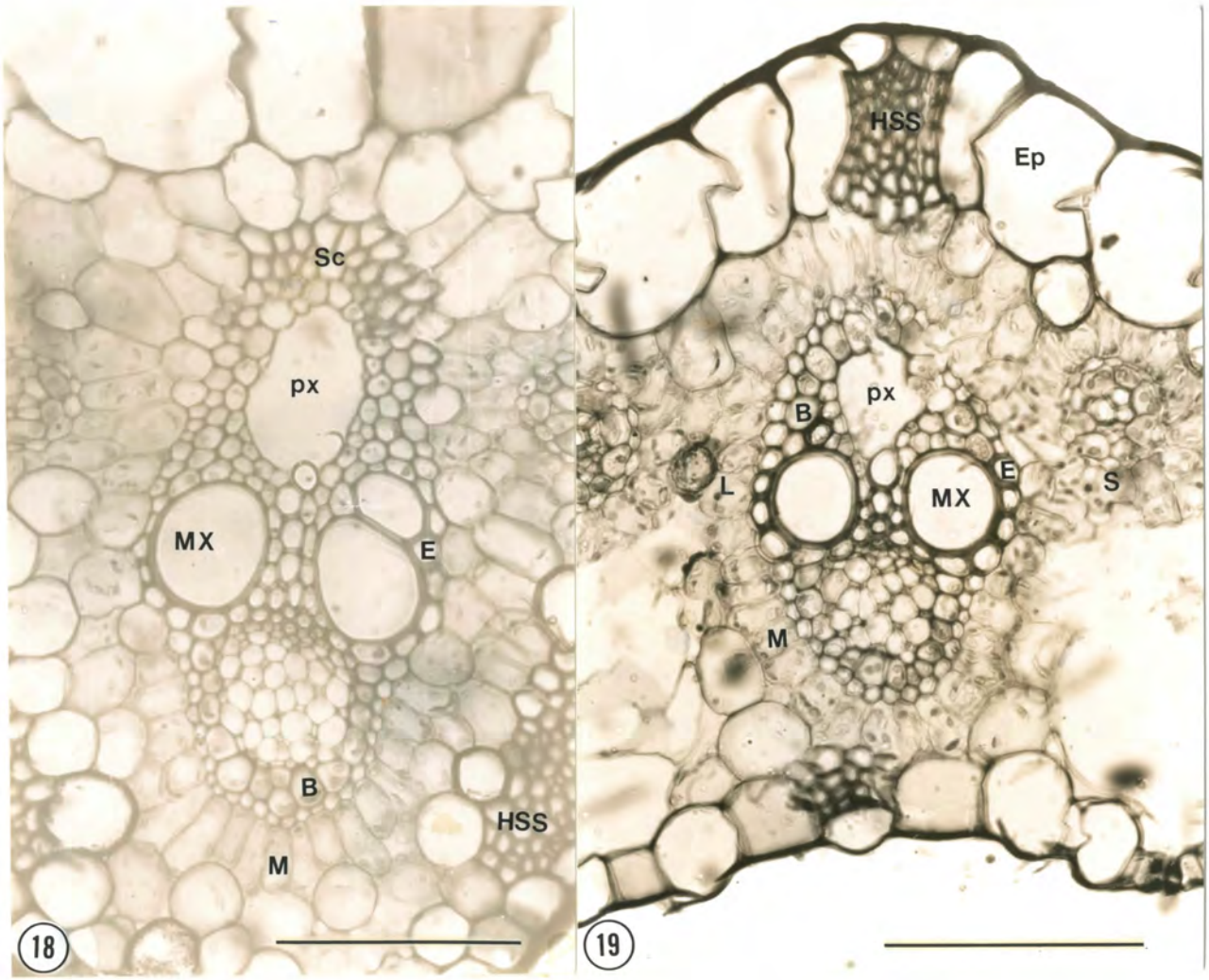
Sclerenchymatous caps are absent from the adaxial side of the large bundles and the radiating mesophyll is entire and not restricted to the phloem side of the vascular bundle (Fig. 19). The large bundle contains two large metaxylem vessels and associated protoxylem lacunae. The Kranz bundle sheath is only interrupted by two large metaxylem vessels and is not restricted to the phloem half of the vascular bundle. The two large metaxylem vessels are bridged by 8 to 10 tracheary elements.

3.2.3.2.3 *Intermediate vascular bundles*

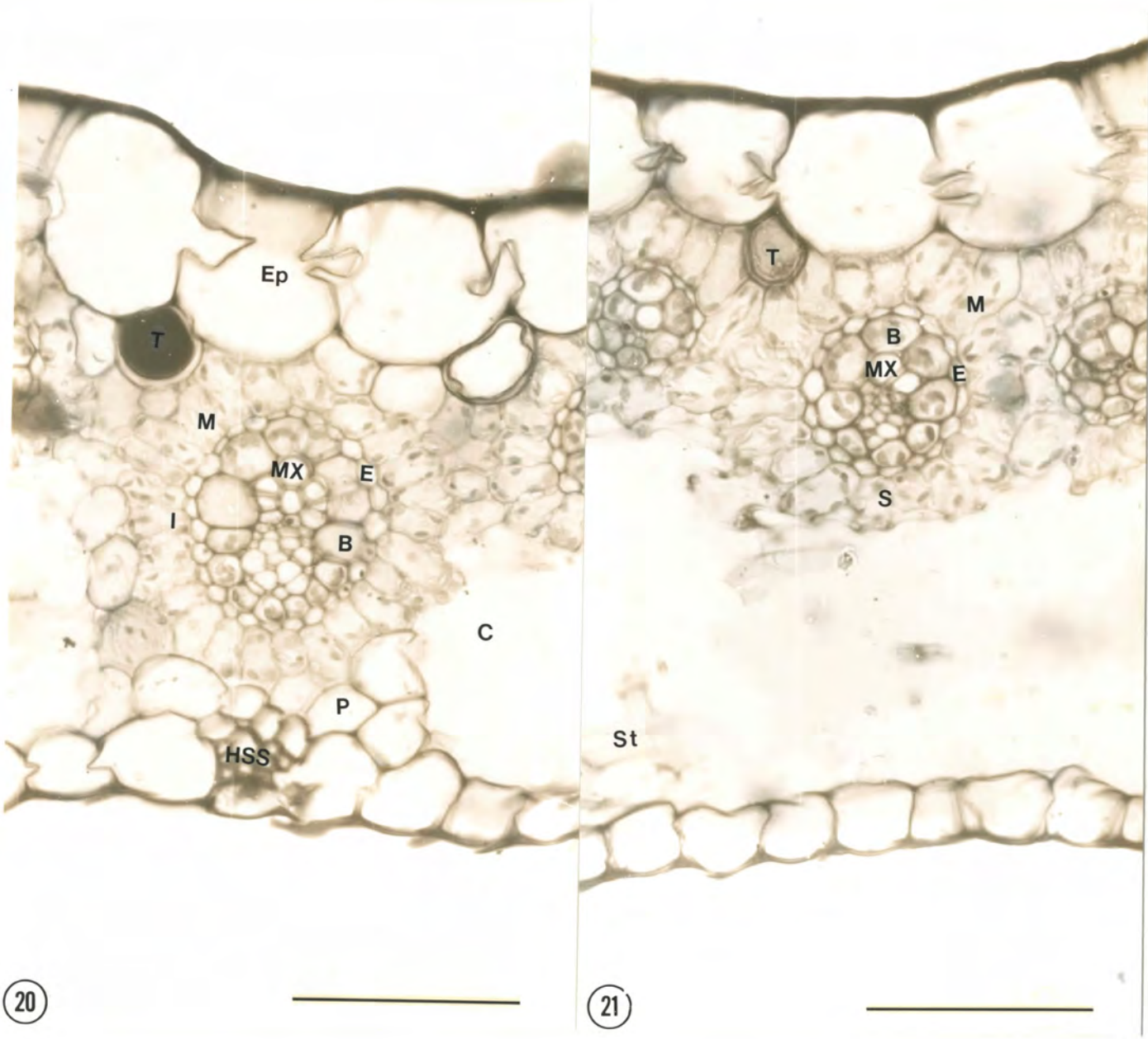
The radiating mesophyll is entire and not restricted to the phloem half of the vascular bundle (Fig. 20). There is no protoxylem lacunae associated with the metaxylem vessels. The Kranz bundle sheath is entire and surrounds the vascular tissue. There are no large metaxylem vessels and the metaxylem vessels are not bridged by tracheary elements.

3.2.3.2.4 *Small vascular bundles*

The radiating mesophyll in the small bundles is entire and not restricted to the phloem half of the vascular bundle (Fig. 21). The Kranz bundle sheath is entire and is not restricted to the phloem half of the vascular bundle. The xylem is composed of a few small metaxylem vessels. No xylem tracheids bridge the metaxylem vessels.



Figs. 18 & 19. Show the midrib and large vascular bundle of the mature leaves of site 3 plants. Fig. 18. Shows the midrib vascular bundle and Fig. 19, shows the large vascular bundle and associated leaf tissues. (B)= Kranz bundle sheath; (C)= air cavity once filled with stellate parenchyma; (E)= mestome sheath; (Ep)= epidermal cell; (HSS)= hypodermal sclerenchymatous strand; (L)= large vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (px)= protoxylem lacunae; (S)= small vascular bundle and (Sc)= sclerenchymatous cap. The bar = 20μm.



Figs. 20 & 21. Show the intermediate and small vascular bundles of the mature leaves from site 3. Fig. 20. Shows the intermediate vascular bundle and associated leaf tissues. Fig. 21. Shows small vascular bundles and associated leaf tissues. (B)= Kranz bundle sheath; (C)= air cavity once filled with stellate parenchyma; (E)= mestome sheath; (Ep)= epidermal cell; (HSS)= hypodermal sclerenchymatous strand; (I)= intermediate vascular bundle; (M)= radiating (Kranz) mesophyll; (MX)= metaxylem vessel; (P)= parenchymatous bridge; (S)= small vascular bundle; (St)= stellate parenchyma and (T)= tannin cell. The bar = 20μm.

3.2.4.1 The Similarities and Dissimilarities between Sites 1, 2 and 3

The leaves of the plants from the three different sites are similar in most respects, sites 2 and 3 (inland sites) are however, the most anatomically similar (Table 1).

Site 1 leaves possess a hypodermis adjacent the ad- and abaxial epidermis. The leaves of sites 2 and 3 however, do not have a hypodermis. The leaves of all three sites are hypostomatous, with the stomata located abaxially, and associated with the stellate parenchyma regions. The stellate parenchyma regions in site 1 leaves are the smallest, followed by site 2 and are the largest in site 3 leaves. Tannin-containing cells are associated with all the vascular bundles of site leaves.

Associated with the large bundles of the leaves from sites 1-3 are ad- and abaxial hypodermal sclerenchymatous strands. The leaves of site 1, differ from sites 2 and 3, in that the hypodermal sclerenchymatous strands are associated with alternate small abaxial bundles. With the exception of site 2, all large bundles have an adaxial hypodermal sclerenchymatous strand associated with them, site 2 leaves have a hypodermal sclerenchymatous strand associated with alternate large bundles. All intermediate bundles have an associated abaxial hypodermal sclerenchymatous strand. Whilst the midrib bundles of site 1 and 2 leaves have two abaxial hypodermal sclerenchymatous strands, site 3 has an additional abaxial, centrally placed strand.

The maximum lateral cell count between all vascular bundles was less than 4 cells. Sections of site 1 leaves contained three rows of vascular bundles, and sites 2 and 3, two rows. The number of small adaxial-situated bundles between the large and intermediate bundles in the lamina of sites 2 and 3 is similar (3-4 and 4-5, respectively), whilst site 1 leaves have 2 ad- and 1 abaxial small bundle between the large and intermediate bundles.

The midrib of sites 1-3 all have a sclerenchymatous cap. The arrangement of the radiating mesophyll in all vascular bundles is the same. The radiating mesophyll in the midribs of sites 2 and 3 is located

only on the phloem side of the vascular bundle and in site 1, the radiating mesophyll is only interrupted by a sclerenchymatous cap. In the large, intermediate and small bundles the radiating mesophyll is entire. The Kranz bundle sheath in the midrib and large bundles is interrupted by large metaxylem vessels and is entire in the intermediate and small bundles except for site 1, where the Kranz bundle sheath of the intermediate bundles is interrupted by two large metaxylem vessels and a protoxylem vessel. In the midrib of sites 1 and 2 leaves, the Kranz bundle sheath is interrupted by two large metaxylem vessels and at site 3 by three large metaxylem vessels. In the midrib bundles of site 3 however, the Kranz bundle sheath was only located on the phloem half of the bundle. The large bundles are interrupted by two large metaxylem vessels at all the sites. The numbers of metaxylem vessels seen in transections of leaves from the three sites varies. Tracheary vessels are present in all midrib and large bundles, and absent in all intermediate and small bundles, with the exception of site 1. The metaxylem vessels in site 1 intermediate bundles are bridged by tracheary elements. Protoxylem lacunae occur in all midrib and large bundles and are absent in all intermediate and small bundles.

In summary the differences between the site leaf anatomy of *M. congestus* are the following: presence or absence of a hypodermis, the arrangement of hypodermal sclerenchymatous strands above the large bundles and below the small bundles, the number of hypodermal sclerenchymatous strands below the midrib, the size of the stellate parenchyma regions, the number of rows of vascular bundles, the arrangement of vascular bundles in the lamina, the number of large metaxylem vessels that interrupt the Kranz bundle sheath and the number of large metaxylem vessels.

Table 1. Summary of the similarities and or dissimilarities between the leaf anatomy of *M. congestus* at sites 1, 2 and 3.

CHARACTERISTICS	SITE No. 1	2	3
Adaxial epidermis	Lc	Lc	Lc
Abaxial epidermis	Sc	Sc	Sc
Hypodermis	p	a	a
Hypodermal sclerenchymatous strand above large bundles	p	alt	p
Hypodermal sclerenchymatous strand below large bundle	p	p	p
Hypodermal sclerenchymatous strand below small bundle	alt	a	a
No. hypodermal sclerenchymatous strands below midrib bundle	2	2	3
Stomata	ab	ab	ab
Tannin cells	with vb	with vb	with vb
Size of stellate parenchyma regions	small	intermediate	large
No. of rows of vascular bundles	3	2	2
Maximal lateral cell count between vascular bundles	<4	<4	<4
No. of small vascular bundles between large and intermediate vascular bundles	2 ad, 1 ab	3-4 ad	4-5 ad
Sclerenchymatous cap on midrib bundle	p	p	p
Radiating (Kranz) Mesophyll			
Midrib bundle	phloem side	phloem side	phloem side
Large bundles	e	e	e
Intermediate bundles	e	e	e
Small bundles	e	e	e
Kranz bundle sheath			
Midrib bundle	i: MX	i: MX	i: MX
Large bundles	i: MX	i: MX	i: MX
Intermediate bundles	i: MX	e	e
Small bundles	e	e	e

CHARACTERISTICS	SITE No. 1	2	3
No. of large metaxylem vessels			
Midrib bundle	4	2	3
Large bundle	2	2	2
Intermediate bundle	2	0	0
Small bundle	0	0	0
Tracheids			
Midrib bundle	p	p	p
Large bundles	p	p	p
Intermediate bundles	p	a	a
Small bundles	a	a	a
Protoxylem lacunae			
Midrib bundle	p	p	p
Large bundles	p	p	p
Intermediate bundles	a	a	a
Small bundles	a	a	a

Abbreviations: **a**, absent; **ab**, abaxial; **ad**, adaxial; **alt**, alternate; **e**, entire; **i:**, interrupted by; **Lc**, large cells; **MX**, large metaxylem vessels; **p**, present; **Sc**, small, compact cells; **vb**, vascular bundle.

CHAPTER 4

The field investigation of gas exchange, light, soil moisture and temperature regimes of *Mariscus congestus* at sites 1, 2 and 3

4.1 INTRODUCTION

The CO_2 assimilation of leaves is affected mainly by the nutrition, light regime, leaf age and soil moisture content of the niche that a plant occupies (Pattey *et al.* 1991; Kaufmann 1982[b]; Von Caemmerer & Farquhar 1981; Berry & Björkman 1980). The net changes in the rate of assimilation by the leaves is however, a reflection of the changes in stomatal conductance (Von Caemmerer & Farquhar 1981) and hence mesophyll conductance of CO_2 (Balasimha *et al.* 1991). The primary factors that control the conductance of stomata are the intensity of light and availability of soil water to the plant (Kaufmann 1982[a]).

Soil drying greatly affects the rate at which plants grow, as well as the rate and pattern of plant development (Davies *et al.* 1990). To a large extent this is because both the growth processes and the process of the regulation of CO_2 fixation are sensitive to a reduction in water availability (Davies *et al.* 1990; Sharkey & Seemann 1989; Björkman & Powles 1984). When there is a reduction in soil water availability, water uptake by the plant is reduced, which results in a reduction of the water content of the plant. This results in a decrease in water potential and the loss of turgor in the leaves. All these factors promote stomatal closure and thus a reduction of the net CO_2 influx (Davies *et al.* 1990; Wise *et al.* 1990). While most of the reduction in CO_2 assimilation is attributed to the stomatal closure, part of the reduction is also attributed to the direct effect of dehydration on the biochemical reactions of photosynthesis (Sharkey & Seemann 1989; Ben *et al.* 1987). Severe osmotic stress of chloroplasts and cells, can inhibit the important photosynthetic enzymes, such as Fructose 1,6-bisphosphatase by the inhibition of the photosynthetic light activation or by the lowering of the chloroplast pH (Sharkey & Seemann 1989). The inhibition of light activation is due to the uncoupling of the photophosphorylation process of the electron transport system, rather than from light-dependant damage of photosystem II (Wise *et al.* 1990; Ben *et al.* 1987; Björkman & Powles 1984). The uncoupling of the electron chain leads to an excess of excitation energy in the leaf, resulting in the expression of photoinhibition by the plant and hence causing a reduction in the CO_2 assimilation of the plant (Björkman & Powles 1984). The effects of light on the photosynthetic processes in plants will be dealt with further in Chapter 5.

Plants that are well adapted to minimise water loss relative to carbon gain, possess the C_4 photosynthetic pathway. The ratio of water loss relative to carbon gain is termed water use efficiency (WUE) of that plant and thus the higher WUE by the C_4 plants can be attributed impart to their CO_2 concentrating mechanisms. In general C_4 plants have better WUE than C_3 plants (Trlica & Biondini 1990; Osmond 1987; Boutton *et al.* 1980; Brown & Simmons 1979). C_4 plants are generally more efficient at temperatures higher than 25°C and less efficient at temperatures below 25°C, than C_3 plants (Hatch 1987). While the survival of a plant in its habitat is often considered to be limited by water there is increasing evidence that temperature, through a direct limitation of CO_2 uptake rates can also be significant (Boutton *et al.* 1980; Pearcy 1977[a]).

Observations under both field and laboratory conditions have clearly established that in many species a degree of plasticity or capacity for accimilation exists in the photosynthetic response to temperature (Pearcy 1977[b]). Plants preconditioned in habitat growth temperature regimes or in different seasons exhibit shifts in their photosynthetic response to temperature (Pearcy 1977[b]; Fryer & Ledig 1972). This in many cases appears to be an adaptive response to the environment and results in enhanced CO_2 fixation in that habitat (Read 1990; Pearcy 1977[b]). The effect of temperature on photosynthesis will be discussed further in Chapter 5.

4.2 RESULTS

Site 2 plants reached the end of their growth period (died-back) during November 1990, regrew in February 1991. The absence of field gas exchange results at site 2 during the month of January 1991 was due to a fire that damaged the area (Fig. 22). The plants at site 3 died-back in September 1990 and regrew in October 1990. The death of the site 3 plants at the end of the investigation period was due to a severe and prolonged drought rather than the annual die-back of the species.

Fig. 22. Shows the condition of the vegetation at site 2 after the veld fire on the 15th of January 1991, which made further data collection at this site impossible for January 1991.

4.2.1 Site Climatic conditions

4.2.1.1 *Light intensities*

The light intensity of each site was investigated only during the periods when *Mariscus congestus* was growing and thus there are no light intensity values for those sites experiencing die-back.

The average monthly light intensity at each of the sites indicated that the light environment for each site was different (Fig. 23, Table 1, Appendix 1). The average daily light intensity recorded monthly at each site over the one year period indicated that site 1 received the highest light intensities, site 2 intermediate light intensities and site 3 the lowest (973.68±277.11µmol $m^{-2}.s^{-1}$; 479.06±226.43µmol $m^{-2}.s^{-1}$.; 443.31±224.22µmol $m^{-2}.s^{-1}$ respectively). The high daily incident light intensities investigated monthly at site 1 was due to the absence of shading by other plants within a radius of 5m. The high light intensity of this area may have contributed to the high CO_2 assimilation rates, transpiration rates, stomatal conductance, and WUE of the site 1 plants. The periods of highest average monthly light intensity was recorded at site 1 during October 1990 (1308.33µmol $m^{-2}.s^{-1}$). The highest average monthly light intensity recorded during the investigation period at all the sites was in October 1990

(877.25µmol $m^{-2}.s^{-1}$) and February 1991 (729.11µmol $m^{-2}.s^{-1}$). The light environment of sites 2 and 3 have similar light intensities even though their natural environments were different. Site 2 was located in an exposed hill-side habitat and site 3 in a shaded habitat. The site light intensities were however, all significantly different (Chi-square contingency table testing and Students t-test for two sample means).

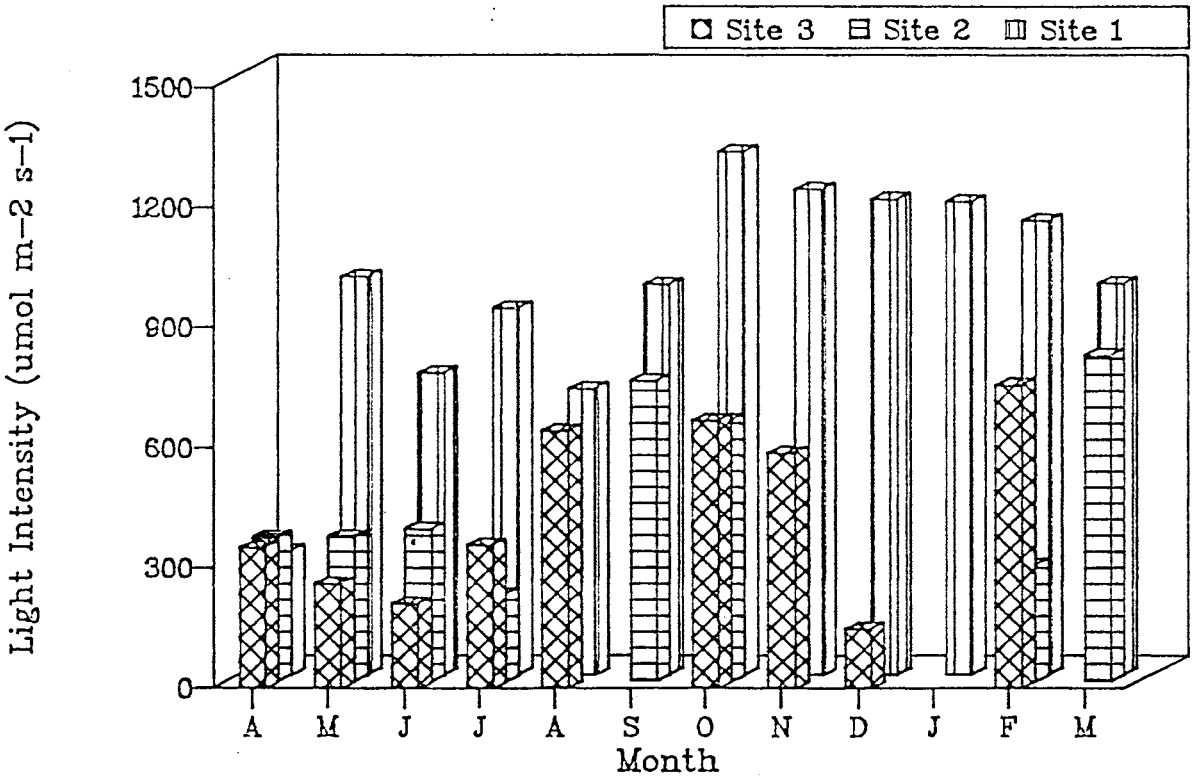


Fig. 23. Shows the average daily light intensity measured for one day of each month for sites 1 to 3, from April 1990 to March 1991.

4.2.1.2 *Soil moisture content*

The periods of highest soil moisture content were recorded during April to May 1990 (Fig. 24, Table 2, Appendix 1). The moisture content of the soils then decreased until December 1990 due to a severe prolonged drought in the Eastern Cape. The soil moisture content after December 1990 increased progressively during January to March 1991, due to steady rains falling in the investigation areas. The

periods of highest soil moisture at each site, thus correlates with the periods of rainfall in this area. The highest monthly soil moisture content was recorded at site 2 in May 1990 (21.31%) and the lowest at site 1 during December 1990 (0.23%). The mean yearly soil moisture contents were as follows: site 2 the highest (12.45±6.56%), site 3 intermediate (5.50±3.20%) and site 1 the lowest (2.19±1.34%). The high moisture content of the soils at site 2 (Katspruit soil form) may have been due to the high clay content of the soil, which correlate with a high water storing capacity. Site 3 experienced low soil moisture contents, which may have been due to the high sand component of its soil (Villafontes soil form), which has a relatively low water storing capacity. Site 1 soil was composed mostly of crushed shells (Fernwood soil form) and its highly exposed nature may have contributed to the extremely low percentage soil moisture recorded at this site. The sites all were significantly different for the monthly soil moisture content as well as for the yearly average value (Chi-square contingency table testing and Students t-test for two sample means, respectively).

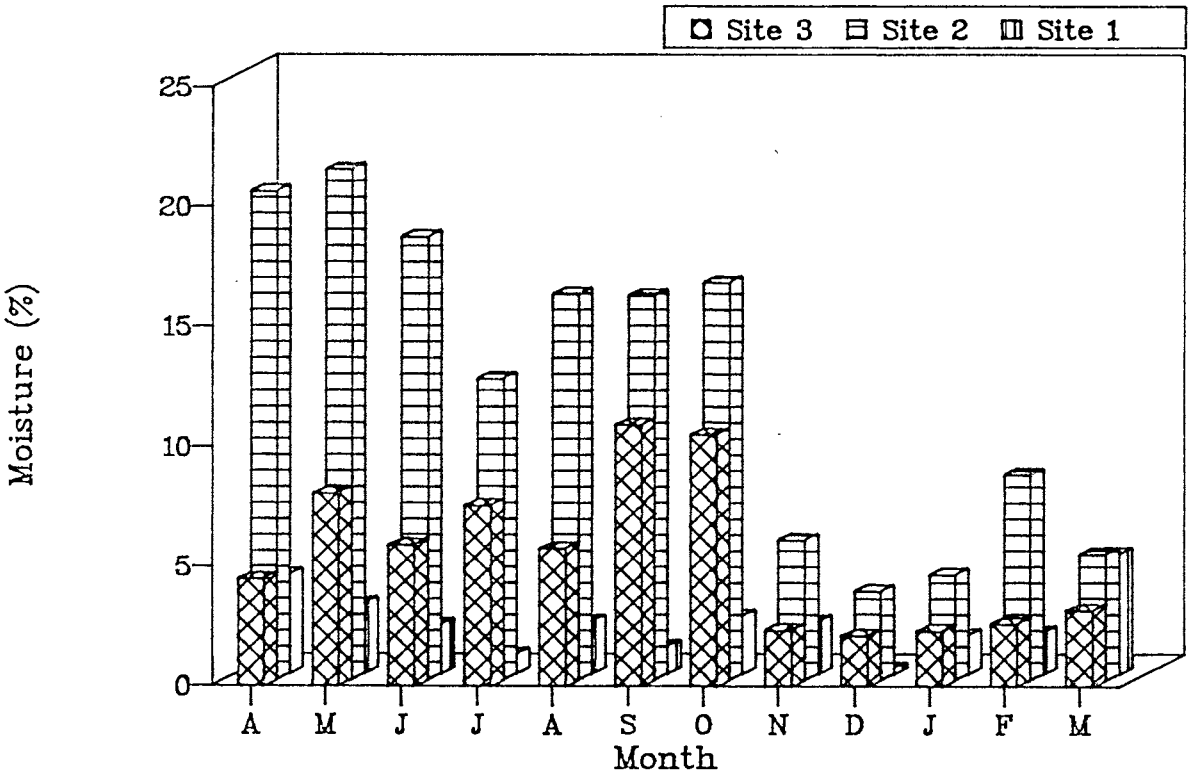


Fig. 24. Shows the average soil moisture content measured for one day of each month for sites 1 to 3, measured from April 1990 to March 1991.

4.2.6.3 *Temperature*

The periods of highest yearly temperature were recorded in January to March 1991 and the lowest in May to July 1990 (Fig. 25, Table 3, Appendix 1). For the sake of clarity, this data was plotted using a line (x-y) graph.

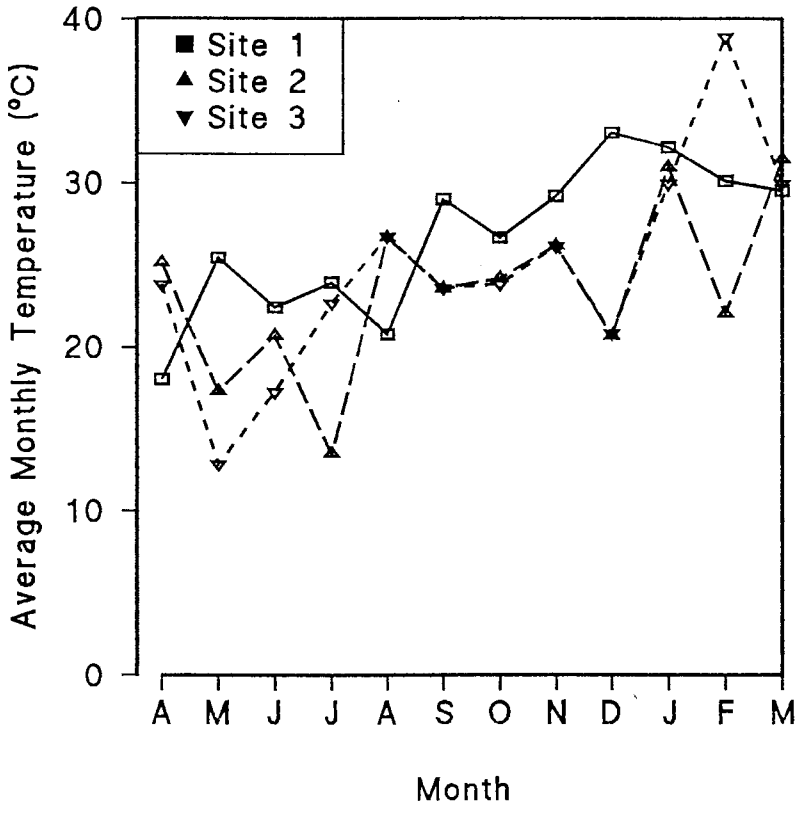


Fig.25. Shows the average temperatures measured for one day of each month for sites 1 to 3, measured from April 1990 to March 1991. The highest and the lowest monthly temperatures were recorded at site 3 in February 1991 and May 1990 respectively.

The highest monthly temperature recorded, was at site 3 in February 1991 (38.97°C) and the lowest at the same site in May 1990 (12.87°C). The average yearly temperatures for the sites were as follows: the highest at site 1 (26.23±4.55°C); site 3 was intermediate (24.75±6.62°C) and site 2 the lowest (23.56±5.20°C). The monthly temperatures of site 1 were significantly different to sites 2 and 3. The temperatures of sites 2 and 3 were however, not significantly different (Chi-square contingency table testing). There was thus a difference between the coastal and inland habitats. The coastal *Mariscus*

congestus plants experienced higher temperatures compared to the inland plants. The yearly averages however, suggested that all the sites were not significantly different in their yearly temperature environments (student t-test for two sample means).

4.2.2 **Monthly net CO_2 assimilation rates**

Site 1 leaves attained the highest average daily CO_2 assimilation rates recorded over the one year period ($17.57\pm6.45\mu mol\ m^{-2}.s^{-1}$) of the sites investigated (Fig. 26, Table 4, Appendix 1). The lowest assimilation rates were attained by site 3 leaves ($2.36\pm1.92\mu mol\ m^{-2}.s^{-1}$) and intermediate CO_2 assimilation rates by site 2 ($4.86\pm3.12\mu mol\ m^{-2}.s^{-1}$). The period of maximal assimilation during the year was during August 1990 to October 1990 and December 1990 to March 1991. The month of maximal assimilation during the yearly period was attained in February 1991, at site 1 ($27.75\mu mol\ m^{-2}.s^{-1}$).

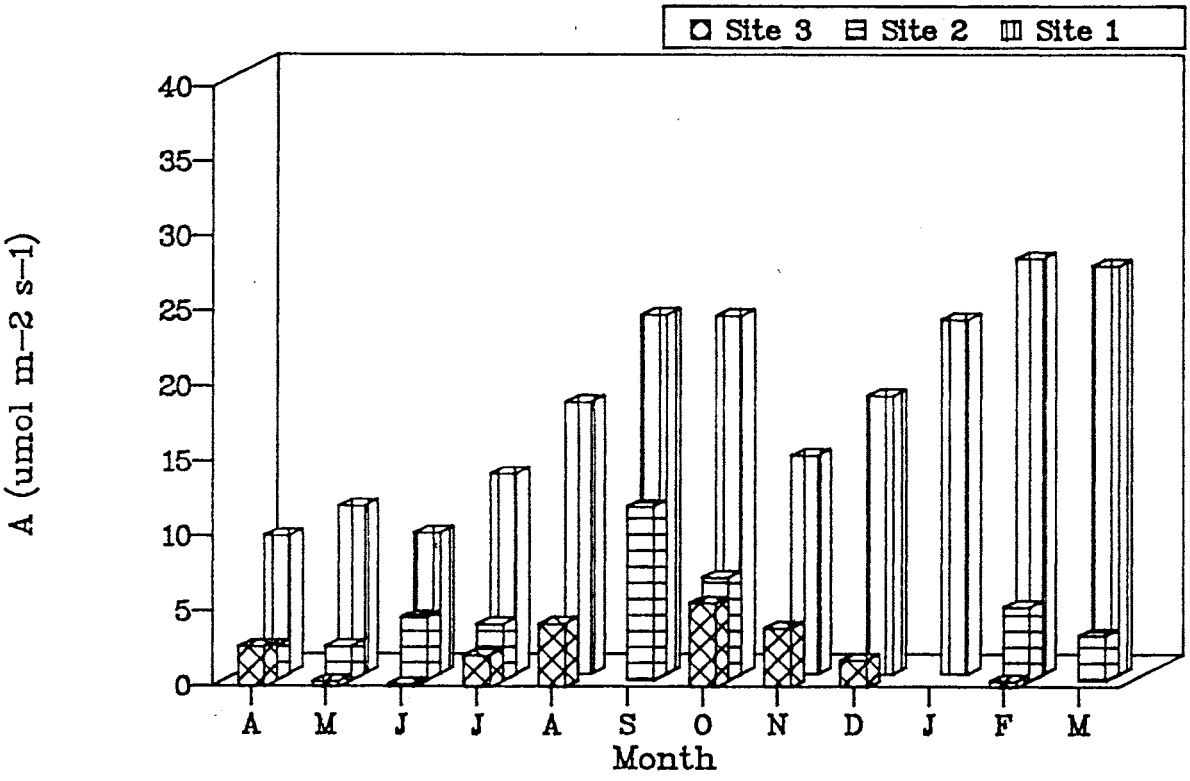


Fig. 26. Shows the average net CO_2 assimilation rates, measured for one day of each month for sites 1 to 3, from April 1990 to March 1991.

The mean yearly assimilation rates of the site 1 leaves were significantly different to those of sites 2 and 3 (Chi-square contingency table testing). Site 2, yearly average assimilation rates were also significantly different to site 3. The field assimilation rates thus suggest that there were differences between the site CO2 assimilation rates at each habitat in response to the habitat climatic conditions.

4.2.3 **Monthly transpiration rates**

The highest transpiration rates over the one year investigation period was attained by site 1 (Fig. 27, Table 5, Appendix 1) and the highest rates recorded for a month was in March 1991 (16.64μmol $m^{-2}.s^{-1}$) by this site. The lowest transpiration rates were attained by site 3 leaves (0.36mmol $m^{-2}.s^{-1}$) in June 1990. The periods of highest transpiration were during September 1990 to October 1990 and January 1991 to March 1991. The average transpiration rates recorded for a month over the one year investigation period at the individual sites were as follows: 9.68±4.48mmol $m^{-2}.s^{-1}$ (site 1); 4.49±2.86mmol $m^{-2}.s^{-1}$ (site 2) and 3.91±2.42mmol $m^{-2}.s^{-1}$ (site 3). The monthly transpiration rates at each site were significantly different (Chi-square contingency table testing).

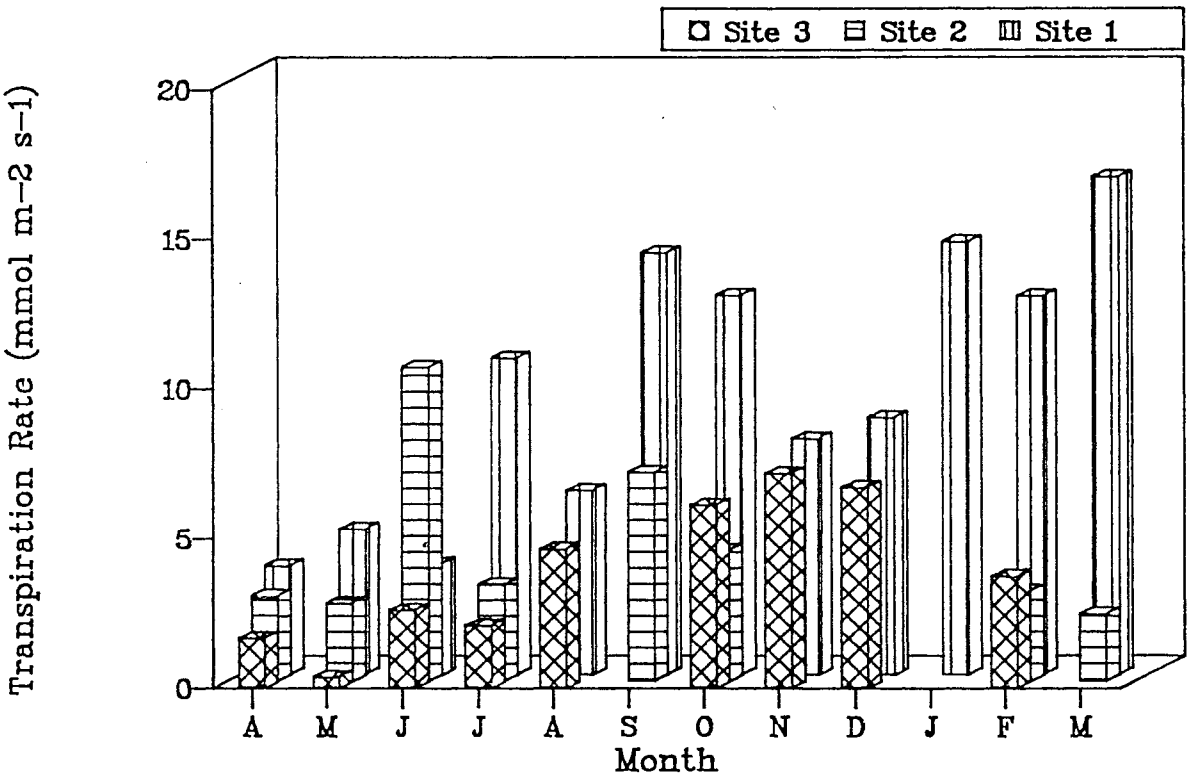


Fig. 27. Shows the average transpiration rates measured for one day of each month for the leaves at sites 1 to 3, from April 1990 to March 1991.

4.2.4 Water use efficiency (WUE)

Site 1 had the highest WUE ($1.90\pm1.40x10^{-3}$mol.mol^{-1}), and site 3 the lowest ($4.50\pm6.10x10^{-4}$mol.mol^{-1}) (Fig. 28, Table 6, Appendix 1). Site 2 had an intermediate WUE ($9.20\pm8.70x10^{-4}$mol.mol^{-1}) (Fig. 28, Table 6, Appendix 1). The highest WUE occurred during April 1990 to June 1990 and lowest during July 1990 to September 1990. The WUE from September 1990 to March 1991 was constant for site 1. The WUE at site 3 decreased, whilst site 2 remained constant during this period, except during the die-back period in November 1990 to January 1991.

The site 1 WUE appears to correlate with the transpiration rate (for the first half of the year). The concomitant increase in assimilation with transpiration decrease in the second half of the year, explains the almost constant WUE of these plants from August 1990 to March 1991. No correlation between WUE and light intensity at this site could be determined. In addition the absence of a significant difference in the WUE from August 1990 to March 1991, may be correlated to an increase in temperature and lowered soil moisture content during this period. The plant thus attained its highest WUE under stress, which is characteristic of C_4 plants (Furbank & Hatch 1987; Pearcy & Ehleringer 1984; Teeri *et al.* 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977).

The WUE at site 2, correlates to soil moisture availability. During periods of high soil moisture content, WUE was high. A possible correlation exists between WUE, light intensity and temperature up to August 1990, after which no apparent correlation between light intensity, temperature or WUE exists. Water use efficiency at site 2 seems therefore to correlate with soil moisture availability, rather than to any other microclimatic variable.

Site 3 was similar to site 2, in that an erratic WUE is demonstrated for the year. Site 3 plants were however, similar to site 1 plants in that the periods of highest WUE occurred during conditions of low soil moisture content, and during the first half of the year (until August 1990) high WUE correlated

with light intensity and temperature. A more complex interaction between the physical environment and WUE therefore seems to occur in site 3 plants compared to sites 1 and 2.

However, for all sites the monthly WUE was significantly different between sites (Chi-square contingency table testing). Even though there appears to be a difference in the monthly WUE shown in Fig. 28, the average yearly WUE of the three sites was not significantly different (Students t-test for two sample means).

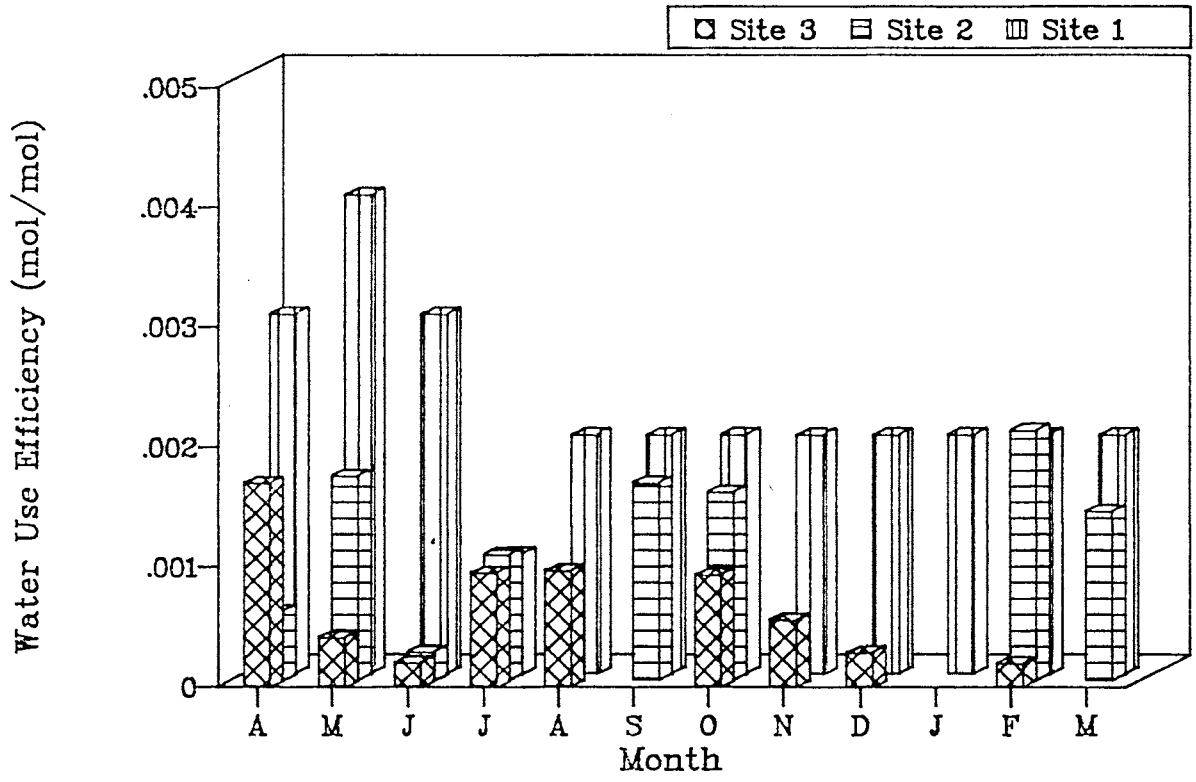


Fig. 28. Shows the average water use efficiency (WUE) measured for one day of each month for the leaves at sites 1 to 3, measured monthly from April 1990 to March 1991.

4.2.5 Stomatal conductance

The highest average monthly stomatal conductance (Fig. 29, Table 7, Appendix 1) was attained by site 2 leaves during June 1990 (945.69mmol $m^{-2}.s^{-1}$). The highest average stomatal conductances recorded once a month over the one year investigation period was however, recorded at site 1 and the lowest at site 3. The average stomatal conductances recorded once a month over the one year investigation period were as follows: site 1: 433.72±251.21mmol $m^{-2}.s^{-1}$; site 2: 293.89±282.18mmol $m^{-2}.s^{-1}$ and site 3: 233.94±144.31mmol $m^{-2}.s^{-1}$.

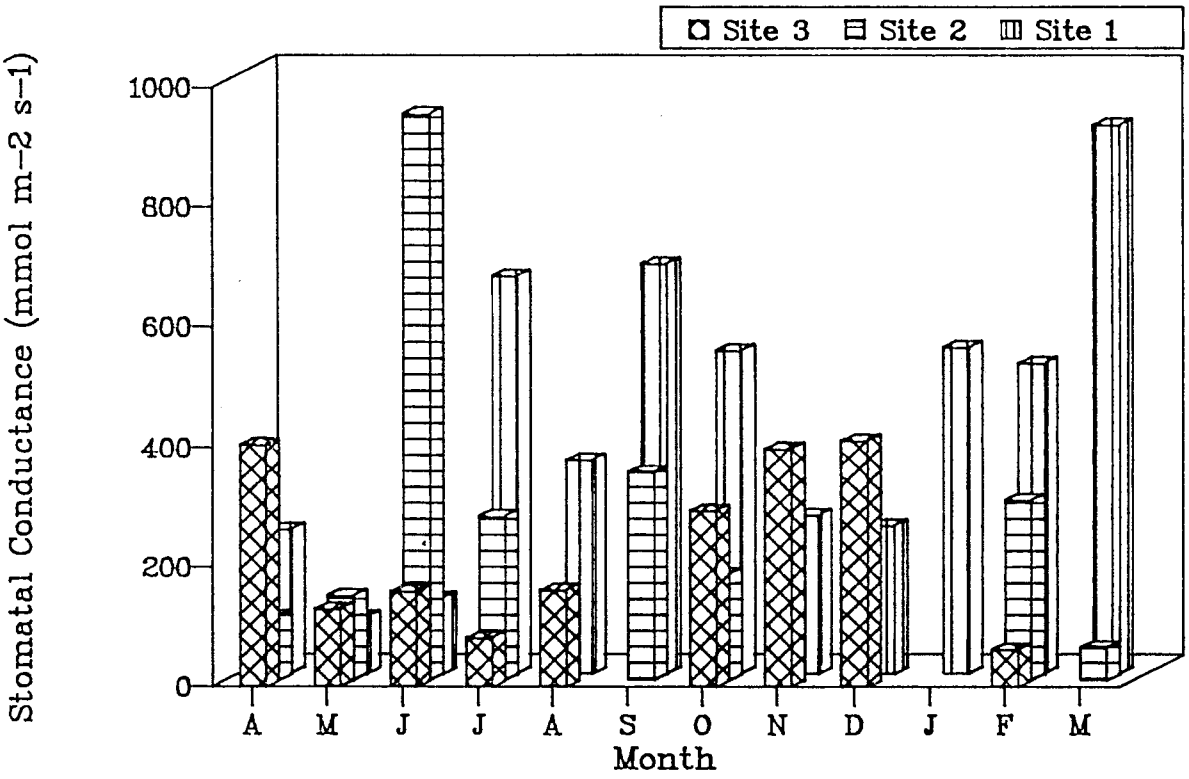


Fig. 29. Shows the average stomatal conductances measured for one day of each month for the leaves of *Mariscus congestus* at sites 1 to 3, measured monthly from April 1990 to March 1991.

The results reflect an overall yearly pattern for the monthly data, in that site 1 had the highest CO_2 assimilation rates and transpiration rates, which may have been due to its high stomatal conductance

rates. And site 3 had lower CO_2 assimilation rates and transpiration rates due to its lower stomatal conductance rates. The stomatal conductances for sites 1, 2 and 3 were significantly different (Chi-square contingency table testing and Students t-test for two sample means).

4.2.7 Summary of monthly field investigations

The environmental climatic characteristics recorded at sites 1, 2 and 3, suggest that the light and soil water regimes were different (Tables 1-3, Appendix 1). The months of highest WUE at sites 1 and 3 correlated with conditions of low soil moisture content, as opposed to site 2 where WUE correlated with conditions of high soil moisture content. Site 3 plants however, appear to have a more complex regulation of WUE, than sites 1 and 2. The high average WUE of site 3, correlated with conditions of high temperature and light intensities, whilst no correlation between the WUE of sites 1 and 2, was apparent under high light intensity and temperature conditions. The monthly temperatures suggest that there was a difference between the coastal and inland temperatures (Table 3, Appendix 1), with the coastal *Mariscus congestus* plants experiencing higher temperatures than the inland sites. There was however, no significant difference between the yearly average temperatures prevailing at each site. The environmental climatic parameters recorded at each site suggest that the conditions at site 1 were harsh. Site 1 experienced the highest light intensities, temperatures, and lowest soil moisture contents. Site 2 had waterlogged soils, intermediate light intensities and temperature conditions. Site 3 had the lowest light intensities, temperature regimes and intermediate soil moisture contents.

The gas exchange characteristics recorded at each site suggest that the CO_2 assimilation, transpiration and stomatal conductance was different (Tables 4-7, Appendix 1). There was however, no significant difference between the site WUE. Site 1 in response to its harsh conditions had the highest assimilation rates, transpiration, stomatal conductances, and WUE. This may thus suggest that the coastal *M. congestus* plants have adapted well to their harsh environmental conditions. Site 3 had the lowest assimilation rates, transpiration rates, stomatal conductances, and WUE. Site 2 was intermediate to sites 1 and 3 in response to its waterlogged, low light environment.

CHAPTER 5

The temperature, light intensity, CO_2 and O_2 concentration response of site 1-3 leaves

5.1 INTRODUCTION

In any ecophysiological investigation it is important to compare field and laboratory based gas exchange studies. A field study enables the investigator to establish what environmental factors the plant has to endure in its natural habitat and how the plant responds to these factors (Chapter 4). Photosynthesis in plants both in the field and the laboratory is controlled by the following important factors namely temperature, light, carbon dioxide, oxygen, nutrient status and watering regime in the leaf tissues (Von Caemmerer & Farquhar 1981). It is thus important for laboratory based investigations to study the gas exchange characteristics of plants, in response to differing regimes of temperature, light, carbon dioxide and oxygen concentration.

5.1.1 Temperature

All enzyme-catalyzed reactions such as photosynthesis are influenced by temperature. The protein nature of enzymes causes them to be sensitive to temperature changes and thus confines their activities to narrower ranges than would generally be encountered in chemical reactions (Devlin & Witham 1983). Extremely high temperatures lead to enzyme denaturisation (Devlin & Witham 1983; Berry & Björkman 1980). In most cases enzyme denaturisation begins at 35°C and is complete at 60°C (Devlin & Witham 1983).

Increasing leaf temperature under atmospheric conditions results in a higher solubility ratio of O_2:CO_2 in the leaf tissues. The higher solubility ratio of O_2:CO_2 will result in the preferential use of oxygen by Ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) even at low oxygen concentrations (Furbank & Hatch 1987; Hatch 1987; Jenkins *et al.* 1987; Ehleringer & Björkman 1977). The C_4 CO_2 concentrating mechanism however, provides a favourable CO_2:O_2 ratio, even at high temperatures. Thus elevated levels of oxygen do not reduce the photosynthetic production of the C_4 plant (Hatch 1987; Ku & Edwards 1978). The favourable ratio of CO_2:O_2, thus permits C_4 plants to proportionally increase their assimilation rates with increasing temperatures. As a result, photosynthesis in C_4 plants is optimal at temperatures above 30°C and 35°C (Devlin & Witham 1983; Berry & Björkman 1980). C_3 plants on

the other hand do not have the C_4 CO_2 concentration mechanism and are inhibited by high temperatures due to an unfavourable O_2:CO_2 ratio in their mesophyll cells. C_3 plants suffer from a high degree of photorespiration, even at the moderate temperatures of 25 to 30°C (Devlin & Witham 1983). C_4 plants thus have a higher potential for photosynthesis at high temperatures than their C_3 counterparts (Hatch 1987; Pearcy & Ehleringer 1984; Berry & Björkman 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977; Gutierrez *et al.* 1974).

5.1.2 **The role of light in photosynthesis**

CO_2 fixation in the mesophyll of C_3 and C_4 plants is light activated. The extent of this activation depends mainly on the light intensity incident on the leaf surfaces. Complete activation in C_4 plants requires a light intensity which approaches at least half full sunlight (Grammatikopolous & Manetas 1990).

Light also drives many of the important processes needed for photosynthesis to occur. It provides energy in the form of ATP, via the photosystem (PS) II cyclic and non-cyclic photophosphorylation electron chain for the Reductive Pentose Phosphate (RPP) pathway (Usuda *et al.* 1984). Light also provides reductive power in the form of NADPH (Edwards *et al.* 1985).

5.1.2.1 ***The light activation of important photosynthetic enzymes and intermediates in CO_2 assimilation***

Light is involved in the regulation of the activity of phosphoenolpyruvate (PEP) carboxylase (Leegood & Von Caemmerer 1989; Usuda *et al.* 1984). The activity of PEP carboxylase is controlled by the light modulation of pyruvate, Pi dikinase (PPDK) which consumes pyruvate to regenerate PEP (Edwards & Ku 1990; Grammatikopolous & Manetas 1990; Leegood & Von Caemmerer 1989; Edwards *et al.* 1985; Usuda *et al.* 1984). Light is also involved in the regulation of pyruvate uptake into the stroma of the mesophyll chloroplasts. In this manner, light controls the pools of a few important C_4 photosynthetic intermediates. A decreased production of PEP would also result in decreased rates of photosynthesis

and hence 3-PGA production. The decreased 3-PGA production would in turn result in a decreased rate of 3-PGA export from the bundle sheath cells to the mesophyll. 3-PGA in mesophyll cells is converted into Triose phosphate, which is a strong activator of PEP carboxylase. Light thus controls the concentration of PEP, the production of Triose phosphate, and the activity of PEP carboxylase, thereby regulating the CO_2 pumping mechanism of the C_4 pathway (Leegood & Von Caemmerer 1989).

Light is also involved in the regulation of the RPP pathway through the control of Ribulose-1,5-bisphosphate (RUBP) regeneration. Photosynthesis is limited when the RUBP levels are less than twice the saturation point concentration of Rubisco. RUBP levels need to be larger than 1 $mol.mol^{-1}$ to saturate Rubisco. Photosynthesis in C_4 plants and moreover C_3 plants at sub-saturating light intensities, are thus also regulated by the rate of RUBP regeneration and not by the activation state of Rubisco (Arulanantham *et al.* 1990). It is also thought that the activation state of Rubisco may be controlled by the photosynthetic electron transport system (Arulanantham *et al.* 1990; Taylor & Terry 1984; Von Caemmerer & Farquhar 1981). Thus even small changes in light intensity at low photosynthetic photon flux densities will result in large variations of the photosynthetic rates in both C_3 and C_4 plants (Furbank & Foyer 1988). As discussed above there is however, a greater regulation of the C_4 photosynthetic processes by light than in C_3 plants. C_4 plants however, still tend to have a higher potential for photosynthesis at or near light saturation than C_3 plants (Hatch 1987).

5.1.3 The effect of varying carbon dioxide and oxygen concentrations on photosynthesis

5.1.3.1 *The effect of varying the CO_2 concentration on photosynthesis*

The initial effect of increasing the concentration of CO_2 results in the increased activation of Rubisco. There is an almost linear response of CO_2 assimilation rate (A) to an increase in internal carbon dioxide (Ci) concentration (Farquhar & Sharkey 1982). The slope of an A/Ci curve is thus proportional to the maximum activity of Rubisco in the leaf (Von Caemmerer & Edmonson 1986; Taylor & Terry 1984;

Farquhar & Sharkey 1982; Von Caemmerer & Farquhar 1981). If the rate of RUBP carboxylation is increased sufficiently at high concentrations of CO_2, the capacity to regenerate RUBP will become limiting. This capacity for RUBP regeneration depends interalia on the capacity for electron transport (Taylor & Terry 1984; Farquhar & Sharkey 1982). The supply of photochemical energy may however, co-limit the CO_2 assimilation rate at carbon dioxide concentrations of 300µl/l and higher (Krall & Edwards 1990; Taylor & Terry 1984). The demand for photochemical energy in C_4 plants is tightly coupled to the CO_2:O_2 ratio. There is a tight linkage between the quantum yield of electrons from PS II (QYE) and the quantum yield of CO_2 assimilation at increased CO_2 levels (Krall & Edwards 1990).

5.1.3.2 *The effect of oxygen on photosynthesis*

The benefits of the C_4 photosynthetic mechanism compared to that of C_3 plants, derives from the capacity of C_4 plants to concentrate CO_2 at the site of Rubisco (Krall & Edwards 1990). Rubisco in C_4 plants, due to the CO_2 concentration mechanisms is thus able to operate in a high CO_2 environment in the bundle sheath chloroplasts, which reduces the effects of the increasing oxygen concentrations in the air (Furbank & Hatch 1987; Ku & Edwards 1978). Increasing the concentration of oxygen in the mesophyll cells, will thus reduce the CO_2 assimilation rates of C_3 plants (Hatch 1987; Von Caemmerer & Farquhar 1981). C_4 plants due to their oxygen insensitivity will thus tend to have low compensation points (Monson *et al.* 1984; Raghavendra & Das 1976; Downton & Tregunna 1968). The compensation points (τ) of C_4 plants is usually close to $0\mu l.l^{-1}$, compared to C_3 plants, where the τ values are closer to 50µl/l and higher. There are however, plants in the Gramineae and Cyperaceae that deviate from the aforementioned C_3 and C_4 compensation points (Krenzer *et al.* 1975).

5.2 RESULTS

5.2.1 Light and Temperature response

5.2.1.1 *Light and Temperature response of site 1 leaves*

The potted site 1 plants (Fig. 30) attained the highest maximum assimilation rates (A_{max}). Site 1 potted plants appeared to be light saturated, at a light intensity of 2400µmol $m^{-2}.s^{-1}$ at 20, 25 and 35°C. The A_{max} at the optimal photosynthetic temperature (35°C) was 32.83µmol CO_2 $m^{-2}.s^{-1}$.

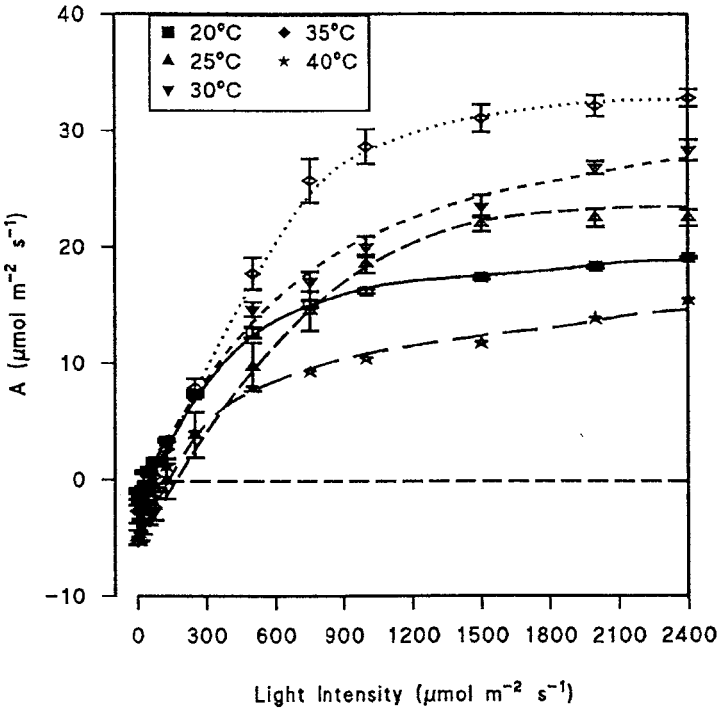


Fig. 30. Shows the light and temperature response of the potted plants from site 1. These plants do not appear to be light saturated even at light intensity of 2400µmol $m^{-2}.s^{-1}$. The optimal photosynthetic temperature of site 1 plants was 35°C.

The laboratory based light and temperature response curves for site 1 potted plants were however, different from the light and temperature response curves extrapolated from the field daily circadian rhythm data (Fig. 31). Whilst the direct extrapolation of data was not feasible due to the many variable changes during the day, the data still can be valuable in the quantitative comparison of the field and

lab gas exchange characteristics. The field results indicated that 25°C was the optimal photosynthetic temperature whilst under field conditions. This suggests that the plants under field conditions have become well adapted to the average yearly temperature of their natural habitat (26.23°C). The field optimal temperature is thus different to the potted plants optimal photosynthetic temperature of 35°C. The A_{max} at the optimal photosynthetic temperature under field conditions was also lower than that of the potted plants (28.08μmol $m^{-2}.s^{-1}$ and 32.83μmol $m^{-2}.s^{-1}$ respectively). The field plants were light saturated at 1500μmol $m^{-2}.s^{-1}$, unlike the potted plants, which were only light saturated at 2400μmol $m^{-2}.s^{-1}$. The field data suggested that there was little deviation in the CO_2 assimilation rates with increasing temperature, which also was different to the potted plants. The potted plants had higher CO_2 assimilation rates than the field plants over similar ranges of light intensities. The gas exchange characteristics whilst under field and laboratory conditions were thus different.

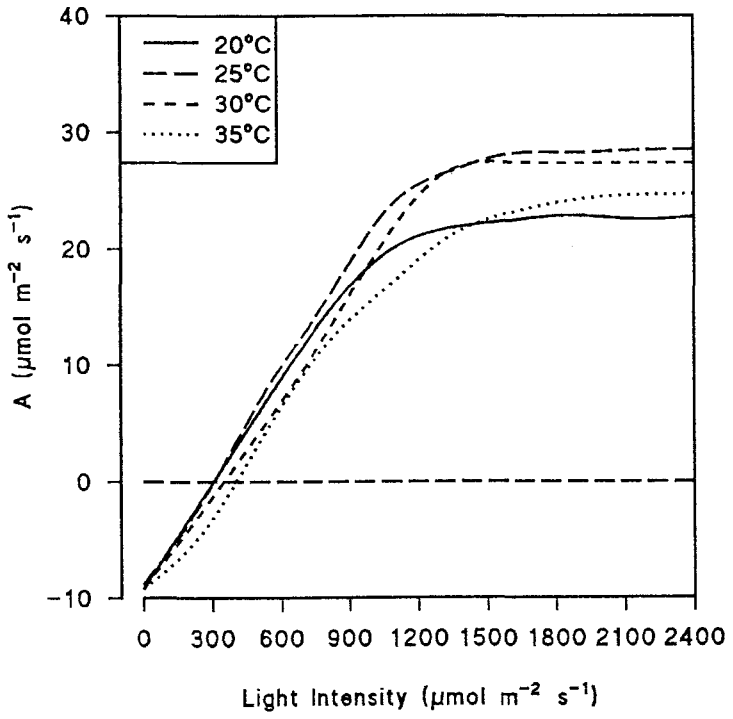


Fig. 33. Shows the light and temperature response curves of *Mariscus congestus* plants at site 1. The curves represent the lines of best fit for data extracted from the daily circadian rhythm CO_2 assimilation rates.

5.2.1.2 *Light and Temperature response of site 2 leaves*

The CO_2 assimilation rates of the potted plants from site 2 were light saturated at a light intensity of 1500µmol $m^{-2}.s^{-1}$, at 20°C and 35°C. At 20 and 35°C, the CO_2 assimilation rates were inhibited at light intensities above 1500µmol $m^{-2}.s^{-1}$. At 25 and 30°C the potted plants of this site were not light saturated, even at a photosynthetic photon flux density (PPFD) of 2400µmol $m^{-2}.s^{-1}$. The potted plants from this site attained their optimal photosynthetic rates (Fig. 32) at 30°C (22.96µmol $m^{-2}.s^{-1}$). Site 2 potted plants (Fig. 37) also had intermediate CO_2 assimilation rates to the potted plants of sites 1 (highest) and 3 (lowest).

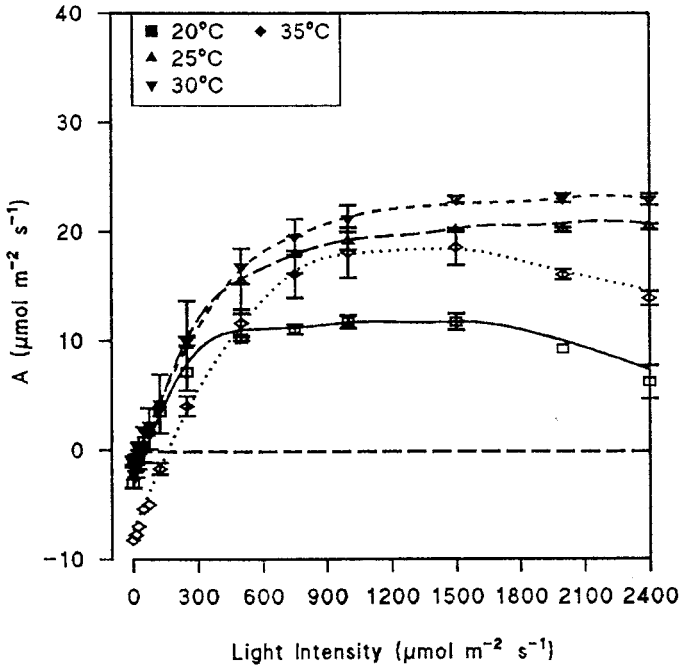


Fig. 32. Shows the light and temperature response for potted plants from site 2. The Figure also illustrates a clear light inhibition and light saturation at a photosynthetic photon flux density (PPFD) of 1500µmol $m^{-2}.s^{-1}$ and above, at 20 and 35°C. No light saturation however, occurs at 25 and 30°C. Optimal CO_2 assimilation occurs at 30°C.

The light and temperature response curves of the potted plants from site 2 differ from the field light and temperature response curves (Fig. 33). The optimal photosynthetic temperature of site 2 field plants was 35°C, which is similar to site 1 and 3 field plants, and was also different to those of the potted plants. The optimal photosynthetic temperatures of the field plants of site 2 (35°C) were however, unlike

site 1 field plants in that their average yearly field temperature was lower (24.75°C) than the optimal photosynthetic temperature. The A_{max} in the field at 30°C and 35°C was higher than the A_{max} attained by the potted site 2 plants (31.54μmol $m^{-2}.s^{-1}$, 29.04μmol $m^{-2}.s^{-1}$ for the field at 30 and 35°C). At 20 and 25°C the plants in the field were light saturated at 900μmol $m^{-2}.s^{-1}$ and their CO_2 assimilation rates were inhibited at light intensities exceeding 1100μmol $m^{-2}.s^{-1}$. The CO_2 assimilation rates were not inhibited by high light intensities at 30 and 35°C. The field plants at these temperatures were however, light saturated at 1500μmol $m^{-2}.s^{-1}$. The field CO_2 assimilation patterns for this site were similar to those obtained for the potted plants at 20 and 35°C. Both the field and potted plants CO_2 assimilation rates were inhibited at 20°C and both were light saturated at 35°C. In the field, site 2 plants attained the highest CO_2 assimilation rates. Site 2 field experiments revealed higher CO_2 assimilation rates than the potted plants from this site. The gas exchange characteristics of the field and potted plants were thus different, except at the temperatures of 20 and 30°C.

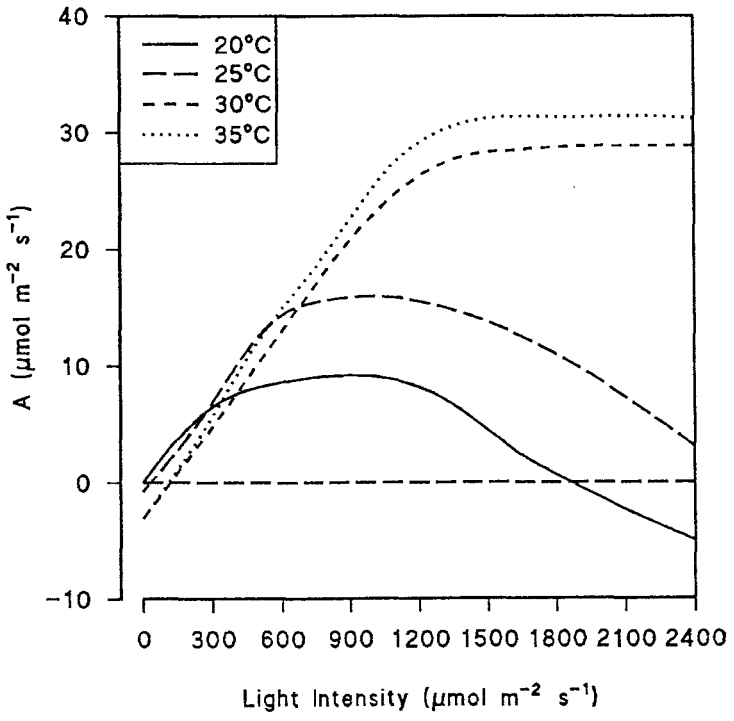


Fig. 33. Shows the light and temperature response curves of *Mariscus congestus* plants at site 2. The curves represent the lines of best fit for data extracted from the daily circadian rhythm CO_2 assimilation rates.

5.2.1.3 *Light and Temperature response of site 3 leaves*

Site 3 potted plants were light saturated at 750μmol $m^{-2}.s^{-1}$ at 25, 30 and 40°C (Fig. 34). Inhibition of CO_2 assimilation at the aforementioned temperatures occurred at a light intensity above 1500μmol $m^{-2}.s^{-1}$, only at 40°C. Site 3 potted plants however, were not light saturated at their optimal photosynthetic temperature (35°C). The potted plants at site 3 had the same optimal photosynthetic temperature (35°C) as site 1 potted plants. The light saturation of site 3 potted plants at 750μmol $m^{-2}.s^{-1}$ may have been due to the adaption of the plants at site 3 to a low light environment, which had an average yearly light intensity of 443.31μmol $m^{-2}.s^{-1}$.

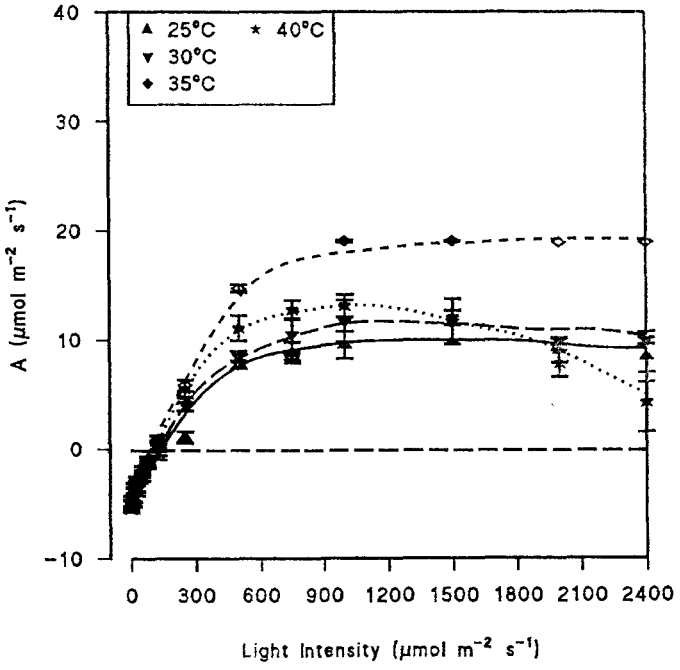


Fig. 35. Shows the light and temperature response curves for the potted plants from site 3. These plants appear to be light saturated above 750μmol $m^{-2}.s^{-1}$. Inhibition of CO_2 assimilation occurs at a light intensity above 1500μmol $m^{-2}.s^{-1}$ at 25, 30 and 40°C. The only temperature that showed no apparent light saturation or inhibition, was the optimal photosynthetic temperature (35°C).

The light and temperature response curves of the potted plants from site 3 differ from the field light and temperature response curves (Fig. 36). These results are thus similar to the responses of the potted and field plants from sites 1 and 2. The optimal photosynthetic temperature of the field plants (30°C)

was also different to the potted plants (35°C), which was similar to the potted plants of sites 1 and 2. Also similar to site 2 and unlike site 1, the field optimal photosynthetic temperature of site 3 plants was different from the average yearly temperature regime of site 3 (approximately 23.56°C). The A_{max} of site 3 field plants at the optimal photosynthetic temperature was also higher than their potted counterparts (26.54μmol $m^{-2}.s^{-1}$ and 18.65μmol $m^{-2}.s^{-1}$ respectively), which was different to site 1 but, similar to site 2. The field plants of site 3 were all light saturated above 1500μmol $m^{-2}.s^{-1}$, unlike their potted counterparts. Similar to site 2, site 3 plants attained higher CO_2 assimilation rates in the field than their potted counterparts under laboratory conditions. The site 3 field data was also similar to site 1 and 2 field plants in that the gas exchange characteristics of field and potted plants were different. The only similarity between site 3 field plants and their potted counterparts was that they both had the lowest CO_2 assimilation rates.

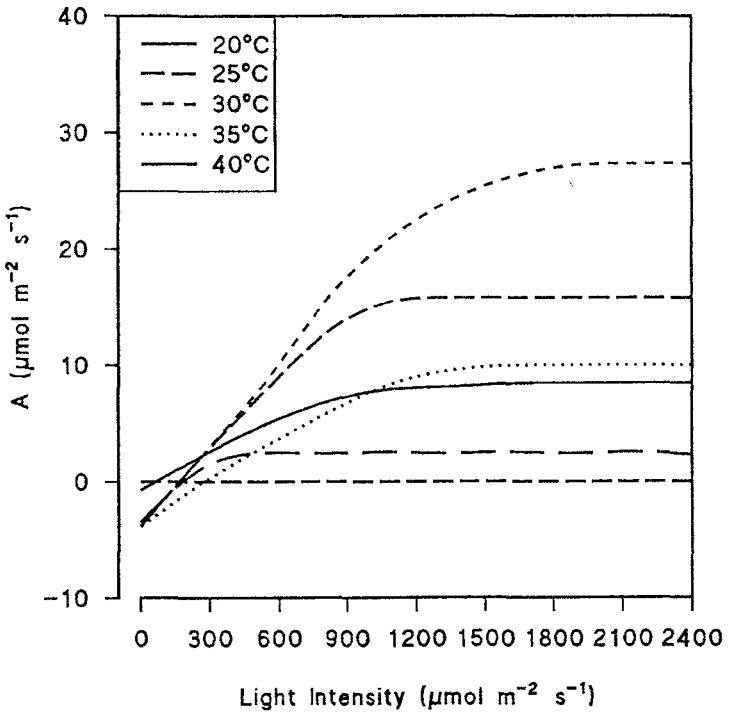


Fig. 36. Shows the light and temperature response curves of *Mariscus congestus* plants at site 3. The curves represent the lines of best fit for data extracted from the daily circadian rhythm CO_2 assimilation rates.

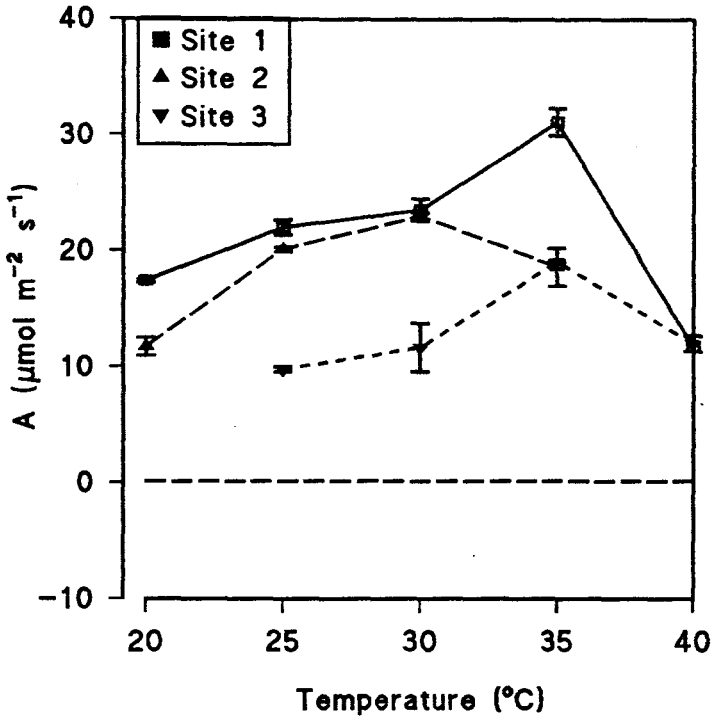


Fig. 37. Shows photosynthetic temperature responses of potted *Mariscus congestus* at 1500µmol $m^{-2}.s^{-1}$ PPFD for sites 1, 2 and 3. Site 1 attained the highest CO_2 assimilation rates and site 3 the lowest.

5.2.2 Oxygen effect

5.2.2.1 *The effect of oxygen concentration on CO_2 assimilation of potted site 1 plants*

The net assimilation rate of site 1 potted plants (Fig. 37) was the highest of all the potted plants at the oxygen concentrations of 0, 8, 16 and 21% (Table 2). The A_{max} of site 1 at 350µl.l^{-1} CO_2, 35°C (optimal photosynthetic temperature) and a PPFD of 1500µmol $m^{-2}.s^{-1}$, were not significantly different for the different oxygen concentrations. The A_{max} values of the potted plants of sites 1 and 2, were far higher than those of site 3. There was no significant difference between the assimilation rates of the site 1 and 2 potted plants. There was however, a significant difference between A_{max} values of the potted plants from sites 1 and 3. The highest A_{max} was attained at 0% and the lowest at 16% oxygen for site 1 plants.

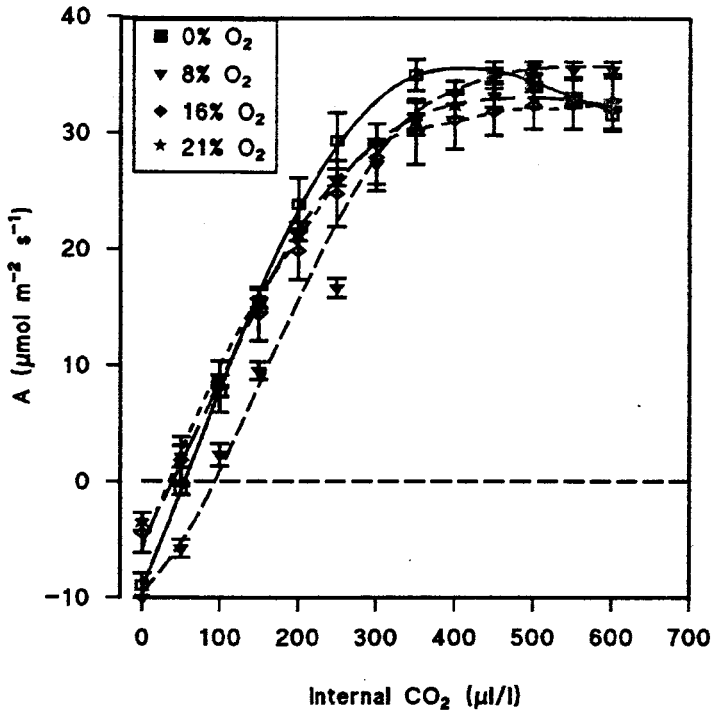


Fig. 37. Shows the effect of oxygen on the net assimilation rate of potted *M. congestus* plants from site 1. Experiments were conducted at 35°C and 1500µmol $m^{-2}.s^{-1}$ PPFD. The highest assimilation rates (35.481µmol $m^{-2}.s^{-1}$) were attained at 8% oxygen and 550µl.l^{-1} CO_2.

Table 2. Shows the net assimilation rates of CO_2 of the potted plants from sites 1, 2 and 3, at 350µl/l CO_2, at each potted plants' respective optimal photosynthetic temperature, 1500µmol $m^{-2}.s^{-1}$ PPFD and 0, 8, 16 and 21% percent oxygen.

Oxygen (%)	A_{max} (µmol $m^{-2}.s^{-1}$)			
	Site 1	Site 2	Site 3	Mean
0	35.00	31.47	19.52	28.66
8	31.23	32.24	18.68	27.83
16	30.10	29.53	22.92	27.52
21	31.00	30.72	19.91	27.21
Mean	31.83	30.99	20.26	

5.2.2.1.1 ***The compensation points of the potted plants from site 1***

The compensation points of the potted plants from site 1 (Table 3) were intermediate to site 2 (lowest) and site 3 (highest). The highest compensation point of the potted plants from site 1 occurred at 8%, and the lowest at 21% oxygen. The compensation points of the potted plants from site 1 showed a definite trend, increasing from 0 to 8%, and decreased from 8 to 21% oxygen. The compensation points of the potted plants from site 1 were not significantly different from those of site 2 and 3 (students t-test for two means). Furthermore there was no significant difference between the different oxygen concentrations for each sites compensation points, except at 8% oxygen.

Table 3. Shows the compensation points of the potted plants from sites 1, 2 and 3, at 0, 8, 16 and 21% oxygen, 1500µmol $m^{-2}.s^{-1}$ PPFD and at each potted plants' respective optimal photosynthetic temperatures.

Oxygen (%)	**Compensation Points (µl/l)**			
	Site 1	**Site 2**	**Site 3**	**Mean**
0	54.71	20.00	91.43	55.38
8	71.77	20.00	64.00	51.92
16	35.29	14.29	64.00	37.86
21	31.77	15.88	75.43	41.03
Mean	48.39	17.54	73.72	

5.2.2.1.2 ***The stomatal limitation on the photosynthesis of the potted plants from site 1***

The stomatal limitations of site 1 potted plants were the lowest (Table 4). The stomatal limitation on the photosynthesis at the different oxygen concentrations were not significantly different for all the site potted plants. Negative stomatal limitation occurred at 16 and 21% for the site 1 potted plants. The calculated stomatal limitations in response to oxygen concentration for potted site 1 plants were significantly different to those of site 2. The stomatal limitations of site 1 potted plants were however,

not significantly different from those of site 3, except at 0 and 16% oxygen (Students t-test for two means).

Table 4. Shows the stomatal limitations on photosynthesis at 0, 8, 16 and 21% oxygen for the potted plants of sites 1, 2 and 3, at each the potted plants' respective optimal temperatures, 1500µmol $m^{-2}.s^{-1}$ PPFD and 350µl/l CO_2.

Oxygen (%)	Stomatal limitation on photosynthesis			
	Site 1	Site 2	Site 3	Mean
0	0.112	0.270	0.025	0.136
8	0.005	0.287	-0.019	0.091
16	-0.033	0.222	0.170	0.120
21	-0.003	0.253	-0.007	0.081
Mean	0.020	0.258	0.042	

5.2.2.1.3 ***The effect of oxygen concentrations on the apparent V_{max} and K_m of the potted plants from site 1***

The $^{app}V_{max}$ and $^{app}K_m$ values of this site are high. These $^{app}V_{max}$ values are however, in a similar range as those found for *Triticum aestivum* by Ku and Edwards (1977). The different oxygen concentrations had a significant effect on apparent V_{max} and K_m values attained by site 1 potted plants (Fig. 38, Tables 5 & 6). The apparent V_{max} values of site 1 potted plants at the different oxygen concentrations, were significantly different (Table 5). The apparent V_{max} of the potted plants from sites 1 and 2 were not significantly different, except at 16 and 21% (students t-test for two means). The highest apparent V_{max} that was attained by site 1 potted plants was at 21% and the lowest at 0% oxygen.

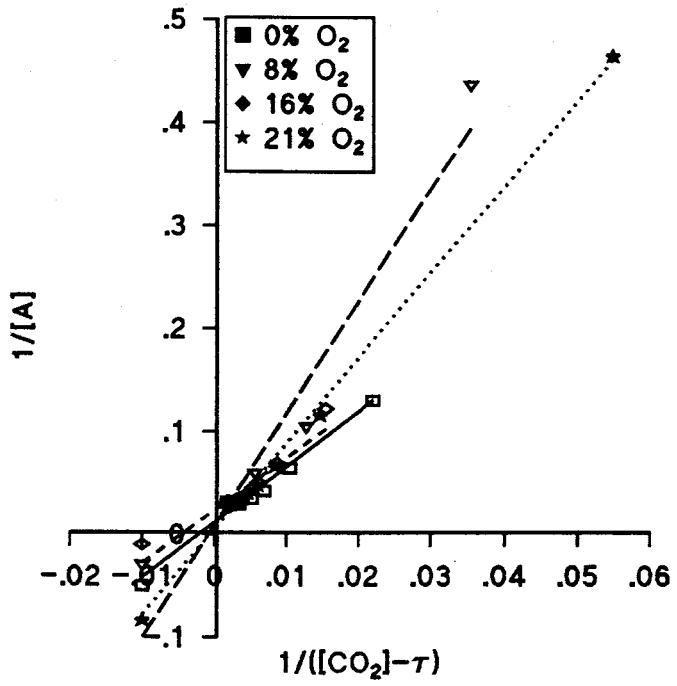


Fig. 38. Lineweaver-Burke plot showing the effect of oxygen concentration on the whole leaf photosynthesis of the potted plants from site 1, at 35°C and 1500μmol $m^{-2}.s^{-1}$ PPFD.

The apparent Km values for the potted plants of the different sites, (Table 6) suggests that effect of the different oxygen concentrations on the apparent Km values were significantly different (students t-test for two means). The apparent Km values of the potted plants from sites 1 and 2, were not significantly different. The highest ^{App}Km value of site 1 potted plants occurred at 21% and the lowest at 16% oxygen.

5.2.2.2 *The effect of oxygen concentration on CO_2 assimilation of potted site 2 plants*

The potted plants of this site had A_{max} values similar to site 1 (Table 2). There was no significant difference between the A_{max} values of site 1 and 2 potted plants (Students t-test for two means). There was also no significant difference between the A_{max} values at 0, 8, 16 and 21% oxygen for this site (Fig. 39) and thus no significant inhibition on CO_2 assimilation by oxygen occurs in site 2 plants. The highest A_{max} of site 2 potted plants was attained at 8% and the lowest at 16% oxygen.

Table 5. Shows the $^{App}V_{max}$ of the potted plants from sites 1, 2 and 3, extrapolated from Fig. 38, 40 and 42. Values were derived from the potted plants' optimal photosynthetic temperatures and 1500µmol $m^{-2}.s^{-1}$ PPFD.

Oxygen (%)	Apparent V_{max} ($\mu mol.m^{-2}.s^{-1}$)			
	Site 1	Site 2	Site 3	Mean
0	85.57	99.79	81.11	88.82
8	121.02	185.05	35.68	113.92
16	43.29	108.83	35.88	62.67
21	200.36	91.95	58.14	116.82
Mean	112.56	121.405	52.70	

Table 6. Shows the $^{App}K_m$ values for the potted plants from sites 1,2 and 3, at 0, 8, 16 and 21% oxygen, extrapolated from Figs. 38, 40 and 42. The tabled responses were attained at each potted plants' respective optimal photosynthetic temperature and 1500µmol $m^{-2}.s^{-1}$ PPFD.

Oxygen (%)	Apparent Km ($\mu l.l^{-1}$)			
	Site 1	Site 2	Site 3	Mean
0	515.32	780.46	830.53	708.77
8	1389.98	1815.67	337.00	1180.88
16	256.88	800.45	300.35	452.56
21	1637.32	610.76	619.50	975.86
Mean	964.88	1001.84	521.85	

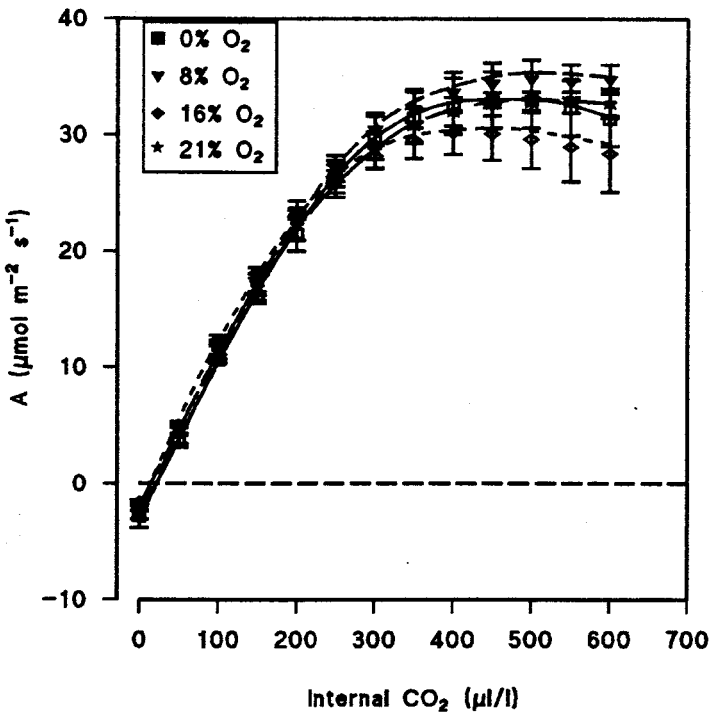


Fig. 39. Shows the effect of 0, 8, 16 and 21% oxygen on the CO_2 assimilation of potted *M. congestus* from site 2. The experiment was conducted at 30°C and 1500µmol $m^{-2}.s^{-1}$ PPFD.

5.2.2.2.1 ***The compensation points of the potted plants from site 2***

The compensation points of these potted plants were significantly lower than those of sites 1 and 3 (Table 3). The general trend of these potted plants was a decrease in compensation point with increasing oxygen concentration up to 16%. The highest compensation point of site 2 potted plants was at 0 and 8% and the lowest at 16% oxygen. There was no significant difference between the compensation points at the different oxygen concentrations of site 2 potted plants.

5.2.2.2.2 ***The stomatal limitation on photosynthesis of the potted plants from site 2***

The calculated stomatal limitation on the assimilation rate of site 2 potted plants at 0, 8, 16 and 21% oxygen, were 450% higher than site 3 and 1000% higher than site 1 potted plants (Table 4). The stomatal limitations of site 2 potted plants were significantly different from those sites 1 and 3. There was however, no significant difference between the stomatal limitations of the potted plants of site 2

to the differing oxygen concentrations. The highest stomatal limitation on photosynthesis of site 2 potted plants occurred at 8% and the lowest at 16% oxygen.

5.2.2.2.3 ***The effect of oxygen concentrations on the apparent V_{max} and Km values of the potted plants from site 2***

The different oxygen concentrations have a significant effect on the apparent V_{max} and Km values of site 2 (Fig. 40). Site 2 leaves similar to site 1 leaves attain high $^{app}V_{max}$ and ^{app}Km values. These values are within range of $^{app}V_{max}$ and ^{app}Km values reported for *T. aestivum* in Ku and Edwards (1977). The $^{App}V_{max}$ at 0, 8, 16 and 21% oxygen concentrations were significantly different (Table 5). Site 2 potted plants, $^{App}V_{max}$ values were higher than those of sites 1 (intermediate) and 3 (lowest). The highest $^{App}V_{max}$ of site 2 potted plants occurred at 8% and the lowest at 21% oxygen.

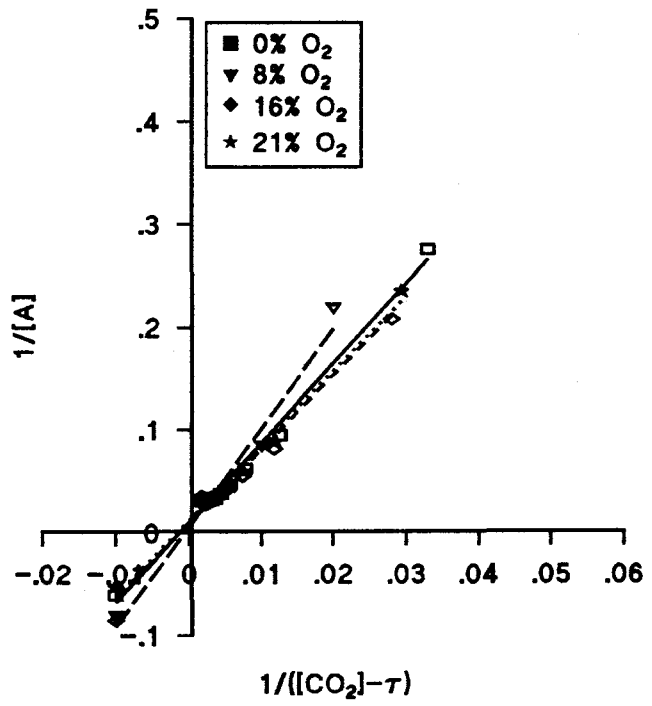


Fig. 40. Lineweaver-Burke plot showing the effect of oxygen concentration on the whole leaf photosynthesis of the potted plants from site 2, at 30°C and 1500μmol $m^{-2}.s^{-1}$ PPFD.

5.2.2.3 ***The effect of oxygen concentration on CO_2 assimilation in potted site 3 plants***

The A_{max} values of the potted plants of this site are the lowest of the sites (Fig. 41, Table 2). The A_{max} values of site 3 were significantly different to those of sites 1 and 2 (Students t-test for two means). There was however, no significant difference between the A_{max} values at the different oxygen concentrations for the potted plants of each site. The highest A_{max} values for site 3 potted plants were attained at 16% and the lowest at 8% oxygen.

5.2.2.3.1 ***The compensation points of the potted plants from site 3***

The compensation points for site 3 potted plants were the highest of all the sites (Table 3). Site 3 compensation points were significantly different to site 2, but were however, not significantly different from those of site 1 (Students t-test for two means). There was no significant difference between the compensation points of the potted plants at the different oxygen concentrations for site 3. The highest compensation point for site 3 potted plants was at 0% and the lowest at 8 and 16% oxygen.

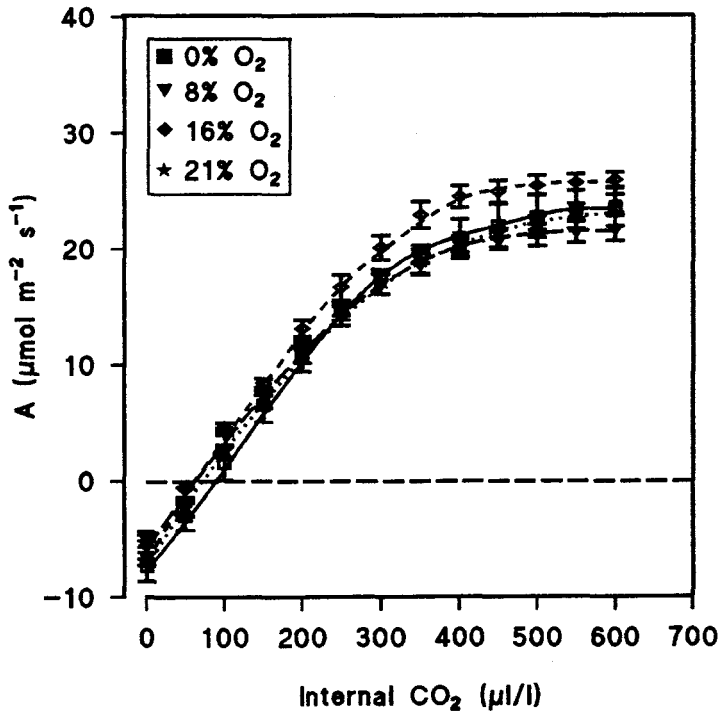


Fig. 41. Shows the effect of oxygen on the photosynthetic rates of potted *M. congestus* from site 3, at 1500µmol $m^{-2}.s^{-1}$ PPFD and 35°C.

5.2.2.3.2 ***The stomatal limitation on the photosynthesis of the potted plants from site 3***

The stomatal limitations of site 3 potted plants were not significantly different from site 1 (Table 4). Site 3 stomatal limitations were however, significantly different to the calculated stomatal limitations on photosynthesis of site 2. The stomatal limitations of the potted plants of site 3 were the highest at 16% and lowest at 8% oxygen. Site 3 potted plants had negative stomatal limitations at 8 and 21% oxygen.

5.2.2.3.4 ***The effect of oxygen concentrations on the apparent V_{max} and Km of the potted plants from site 3***

The $^{app}V_{max}$ and ^{app}Km values of this site are far lower than those of sites 2 and 3. The $^{app}V_{max}$ and ^{app}Km values are however similar to those obtained for *T. aestivum* by Ku and Edwards (1977). The $^{App}V_{max}$ values of the potted plants from site 3 were the lowest of the sites and may suggest why the CO_2 assimilation rates under laboratory and field conditions were low (Fig. 42, Table 5).

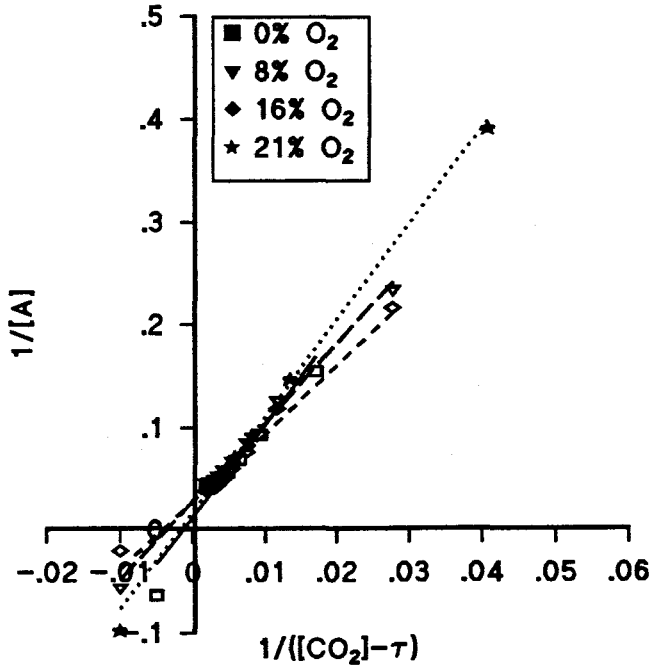


Fig. 42. Lineweaver-Burke regression curves for the oxygen effect on potted *M. congestus* from site 3, at 35°C and 1500μmol $m^{-2}.s^{-1}$ PPFD.

The highest $^{App}V_{max}$ values were attained at 0% and the lowest at 8% oxygen. It is also interesting that the ^{App}Km values of these plants at 8 and 16% were below that calculated for ambient CO_2 levels (Table 6). The highest ^{App}Km values were attained for site 3 potted plants were at 0% and the lowest at 16% oxygen. It is also interesting to note that efficient CO_2 assimilation coupled to lowest photosynthetic inhibition occurred at 0% oxygen. The $^{App}V_{max}$ and ^{App}Km values of site 3 potted plants were also significantly different to those of sites 1 and 2 (Students t-test for two means) (Fig. 43).

5.2.3 Summary of the responses to light, temperature, oxygen and carbon dioxide concentration of sites 1, 2 and 3

5.2.3.1 *Light and Temperature*

Site 1 potted plants had the highest A_{max} values (32.83µmol $m^{-2}.s^{-1}$) at ambient CO_2 and O_2 concentrations and site 3 the lowest. Site 1 potted plants were not light saturated or inhibited at 30, and 40°C, which may have been due to their adaptations to a high light environment habitat. The potted plants of sites 2 and 3 however, were both light saturated and inhibited, which possibly reflects their adaptation to a low light environment habitat. Whereas site 2 potted plants were light saturated at 1500µmol $m^{-2}.s^{-1}$ at 20, 25 and 35°C, site 3 potted plants light saturated at 750µmol $m^{-2}.s^{-1}$ at 25, 30 and 40°C. The potted plants of both site 2 and 3 were inhibited above 1500µmol $m^{-2}.s^{-1}$ PPFD. Site 2 potted plants were inhibited at 20 and 40°C and site 3 at 25, 30 and 40°C. Site 2 and 3 potted plants were not light saturated or inhibited at their optimal photosynthetic temperatures. Site 1 and 3 potted plants both had the same optimal photosynthetic temperature (35°C) whilst site 2 potted plants had a lower optimal photosynthetic temperature (30°C).

The light and temperature response curves of the field plants of each site suggested that the field and potted plants light and temperature responses were different at each site. The optimal photosynthetic temperatures, light saturation points and CO_2 assimilation rates thus were all different. This suggests that the field and potted plants light and temperature responses were not comparable.

5.2.3.2 ***Carbon dioxide and oxygen response of the potted plants from sites 1-3***

CO_2 assimilation, compensation points, and stomatal limitations of the potted plants of sites 1, 2 and 3 were not significantly inhibited by the differing O_2 concentrations. At 0, 8, 16 and 21% oxygen concentrations, site 1 potted plants attained the highest A_{max} and site 3 the lowest. The A_{max} values of the potted plants from site 1 and 2 were not significantly different. Site 3 A_{max} values were however, significantly different to sites 1 and 2.

The highest compensation points were attained by site 3 potted plants and the lowest by site 2. The compensation points at sites 1 and 3 were not significantly different. The compensation points for site 2 potted plants were however, significantly different from sites 1 and 3. Whilst high, the $^{app}V_{max}$ values determined in all cases, are within previously accepted ranges (Ku & Edwards 1977).

The highest stomatal limitations were observed in site 2 potted plants and the lowest in site 1 potted plants. Stomatal limitation of site 2 potted plants were significantly different to those of sites 1 and 3. The stomatal limitation of sites 1 and 3 however, were not significantly different. Both the potted plants of sites 1 and 3 had negative stomatal limitation at 21% oxygen.

The highest $^{App}V_{max}$ was attained by site 2 potted plants and the lowest by site 3. The $^{App}V_{max}$ of sites 1 and 2 were not significantly different. The $^{App}V_{max}$ of potted site 3 plants were however, significantly different from sites 1 and 2.

The highest ^{App}Km was attained by site 2 and the lowest by site 3, but the ^{App}Km values of sites 1 and 2 were not significantly different. The apparent Km values of site 3 potted plants were however, significantly different from sites 1 and 2.

CHAPTER 6

Discussion and Conclusion

6.1 DISCUSSION

6.1.1 Leaf anatomy

The shape of the leaves of sites 1 to 3 were all typically Cyperaceae V-shaped (Metcalfe 1969). Leaves from Site 1 possessed a multi-layered hypodermis, whilst site 2 and 3 plants lacked a hypodermal layer. The development of an ad- and abaxial hypodermis by site 1 leaves may have been an adaptive response to the harsh environmental conditions within which the plants grow at site 1, where the plants were subjected to high salt concentrations that accumulated on their leaf surfaces. Additionally, these plants were also buffeted by abrasive sand-laden winds.

Transections of leaves from all three sites revealed that ad- and abaxial hypodermal sclerenchymatous strands were associated with vascular bundles. Leaves of sites 1 and 2 had two abaxial hypodermal sclerenchymatous strands on either side of the midrib vascular bundles whilst, site 3 leaves had an additional centrally located abaxial strand.

As is typified within the Cyperaceae all leaves contained substomatal air cavities, which were delimited by adaxially-situated stellate parenchyma (Metcalfe 1969). Stellate parenchyma occurred between the large and intermediate vascular bundles at all sites, except site 1 and abaxially below the small bundles. The stellate parenchyma regions of site 1 plants were located between the large and small abaxial bundles and between the intermediate and abaxial small bundles. The arrangement of vascular bundles and stellate parenchyma regions for sites 1-3, is common only to the genus *Cyperus* and *Mariscus* (Metcalfe 1969). In addition sites 1, 2 and 3 also have typical *Mariscus* characteristics, in that their vascular bundles are arranged in two to three rows in the lamina (Metcalfe 1969). The substomatal air cavities in site 1 leaves are smaller than those in site 2 and 3 leaves, which may be an adaptive response to water stress as site 1 leaves are subjected to high salt conditions and low soil moisture. Site 2 plants were relatively waterlogged and the large regions of stellate parenchyma and substomatal air cavities may have assisted not only in transpiration reduction but in effecting favourable CO_2:O_2 exchange rates.

The arrangement of the small adaxial vascular bundles of site 2 and 3 leaves were similar, except that site 3 had four to five small vascular bundles between the large and intermediate vascular bundles and site 2 three to four small bundles. The maximum cell lateral count for sites 1-3, was less than four, indicating that the leaves of *M. congestus* from all three sites were C_4 (Monson *et al.* 1984; Hattersley *et al.* 1982; Hattersley & Watson 1975; Crookston & Moss 1974; Takeda & Fukuyama 1971). The vascular bundle sheath anatomy of the leaf material from all sites, had an outer radiating mesophyll surrounding a mestome or endodermoid sheath, and an inner Kranz bundle sheath, typical of the Chlorocyperiod group within the genus *Mariscus* and *Cyperus* (Ueno *et al.* 1986; Takeda *et al.* 1985, 1980; Hesla *et al.* 1982; Carolin *et al.* 1977; Brown 1975; Laetsch 1974; Metcalfe 1969). The arrangement of chloroplasts within the Kranz bundle sheath was centrifugal, which suggests that all leaves were either C_4 NADP-Me (Pendergast *et al.* 1987; Brown *et al.* 1983; Hattersley & Browning 1981) or PCK (Pendergast *et al.* 1987, 1986; Hattersley & Browning 1981).

The differences in leaf anatomy between the sites, may have been due to the ecotypic divergence of *Mariscus congestus* in its different habitats in response to the differences in plant interactions and microclimate at the different habitats. However, the inherent similarities of the leaves from the different sites, suggest that there are still characteristics that link the these plants at the species level.

6.1.2 **Climatic conditions**

The highest incident light intensity was recorded at site 1 and the lowest at site 3. Sites 2 and 3 (inland sites) had lower temperature regimes than site 1 (coastal site). Site 1 had the lowest soil moisture content and site 2 the highest. On analysis there was no significant difference between the sites at the temperature level. Temperature thus may not exert a large influence on the photosynthetic gas exchange differences recorded both under field and under laboratory conditions. The differences in light and water regimes, are however, a factor of importance, and influence the rate of CO_2 assimilation in plants and hence photosynthetic productivity (Von Kraalingen 1990; Kaufmann 1982[b]; Von Caemmerer & Farquhar 1981).

6.1.3 Field gas exchanges characteristics

Site 1 plants had the highest CO_2 assimilation, transpiration, water use efficiency, and stomatal conductance rates. The high CO_2 assimilation, transpiration and water use efficiency may be due to the accimilation of these plants to the high average yearly light intensity at this site. The high light intensity experienced at this site, may contribute to the high stomatal conductance levels recorded, which would positively influence CO_2 assimilation rates. The higher light intensities may thus have resulted in more energy being available for use in carbon fixation. High light intensities have also been shown to result in higher activities of PEP carboxylase and other important CO_2 assimilation enzymes (Edwards & Ku 1990; Leegood & Von Caemmerer 1989; Edwards *et al.* 1985; Usuda & Edwards 1984). However, high stomatal conductance must result in a higher water loss and hence the high transpiration rates of site 1 plants (Monson & Grant 1989). A high transpiration in a water limiting habitat however, seems illogical, as soil drying must ultimately affect the rate at which plants grow and photosynthesize (Davies *et al.* 1990; Von Kraalingen 1990; Sharkey & Seemann 1989; Meidner 1986; Björkman & Powles 1984). The high transpiration of site 1 plants was however, complimented by concurrent higher CO_2 assimilation rates, which resulted in a higher water use efficiency. Site 1 plants are thus well adapted to their harsh sand-dune habitat and had typical C_4 characteristics since, they had a high potential for photosynthesis under high light intensities and a high WUE, under water limiting conditions (Furbank & Hatch 1987; Pearcy & Ehleringer 1984; Teeri *et al.* 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977; Singh *et al.* 1974).

The lowest CO_2 assimilation, transpiration, stomatal conductance and the lowest water use efficiency was determined in site 3 plants. In all probability, these lower values were in response to the low incident light intensities, and thus the low stomatal conductances recorded. A low stomatal conductance, reduces water loss, thus affecting the transpiration rate and CO_2 assimilation, resulting in the low WUE recorded for the plants at this site. The decreased rate of CO_2 assimilation by site 3 plants may have been as a result of the fact that C_4 plants are uncommon in shaded habitats since, C_4 plants usually require high light intensities for light saturation and high temperature for high photosynthetic rates

(Pearcy & Ehleringer 1984; Boutton *et al.* 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977). The WUE of site 3 however, was influenced by temeprature and soil moisture availability. Site 3 was the only site that displayed typical C_4 characteristics with respect to WUE, since these plants had a high WUE under conditions of high temeprature, light intensity and low soil moisture conditions (Pearcy & Ehleringer 1984; Boutton *et al.* 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977).

Site 2 leaves had intermediate CO_2 assimilation and transpiration rates, a low water use efficiency, and stomatal conductance. The WUE of these plants in response to their habitat conditions was in complete contrast to sites 1 and 3. Site 2 plants had high WUE, whilst under conditions of high soil moisture. This characteristic of site 2 is thus atypical of C_4 plants (Pearcy & Ehleringer 1984; Boutton *et al.* 1980; Doliner & Jolliffe 1979; Ehleringer & Björkman 1977). These intermediate CO_2 assimilation, transpiration and stomatal conductance rates may be attributed to the intermediate light and temperature environment of this site, as well as the waterlogged condition of the soils that these plants grew in since, waterlogged conditions promote stomatal closure, which results in lower CO_2 assimilation and transpiration of that plant (Colin-Belgrand *et al.* 1991; Dreyer *et al.* 1991).

Under field conditions CO_2 assimilation, transpiration and stomatal conductance rates were different for the three sites. Statistically significant differences in gas exchange characteristics were thus attained in response to the habitat microclimate. The differences between the sites thus suggests that whilst under field conditions the sites had ecotypic gas exchange characteristics.

6.1.4 The response of *Mariscus congestus* from sites 1, 2 and 3 to differing light, temperature, CO_2 and O_2 concentrations

6.1.4.1 *Light and Temperature Response for potted plants under laboratory conditions*

Under laboratory conditions, site 1 potted plants had the highest calculated CO_2 assimilation rates of the three sites studied. Potted site 1 plants did not light saturate and were not light inhibited under

laboratory conditions. Sites 2 and 3 however, had lower photosynthetic rates and were light inhibited at high light intensity. This may have been due to the adaptations of sites 2 and 3 to the low light environments of their natural habitat. Plants grown at different light intensities, such as sites 2 and 3 however, usually tend to be light saturated at or near the light intensities prevailing in their natural environments. Plants grown at high light intensities are thus able to attain higher photosynthetic rates per unit area of leaf and are usually only light saturated at higher light intensities than plants grown under lower light intensities (Singh *et al.* 1974). The lack of light saturation by site 1 plants is typical of C_4 plants (Furbank & Foyer 1988; Furbank & Hatch 1987; Doliner & Jolliffe 1979). Sites 1, 2 and 3 all attained optimal photosynthetic temperatures at temperatures above 30°C, which suggests that all the leaves of the sites were C_4 (Devlin & Witham 1983; Berry & Björkman 1980).

6.1.4.2 ***The light and temperature response of the field plants whilst under field conditions***

The light and temperature response curves for the field plants under field conditions for sites 1-3 were different from those obtained for the potted plants under laboratory conditions. This may however, have been due to the diverse nature of the habitats since, it was not possible to investigate gas exchange characteristics under similar watering regimes and months. The results also may not have been comparable due to root binding or a reduced root mass in the potted plants, compared withMthose of the field plants. It may thus have been for the above reasons that the potted and field, light and temperature responses were not comparable.

The field plants under field conditions differed in their optimal photosynthetic temperatures and light saturation points, compared to potted plants from the different sites. A shift in the optimal photosynthetic temperature of plants removed from their natural environment and placed in common environments however is not uncommon as Slayter and Ferrar described in 1977. Site 1 field plants had lower optimal photosynthetic temperatures (25°C) than their potted counterparts (35oC). The differences in optimal photosynthetic temperatures may have occurred due to the field yearly temperatures being too low for optimal photosynthesis to take place. These results are similar to the findings of Slayter and

Ferrar (1977) for *Eucalyptus pauciflora* Sieb. ex Spreng. from different environments grown in a different controlled environment. The field optimal photosynthetic temperature was however, similar to the mean yearly temperature of the site 1 habitat (24.75°C). Site 1 plants had lower CO_2 assimilation rates in the field than their potted counterparts. Sites 2 and 3 field plants however, both had higher CO_2 assimilation rates under field conditions than the potted plants under laboratory conditions.

Site 1 field plants also differed from their potted counterparts, in that they were light saturated at a PPFD of 1500μmol $m^{-2}.s^{-1}$. The yearly mean light intensity of this sites' habitat was 973.68±277.11μmol $m^{-2}.s^{-1}$. The field plants of this site were thus well adapted for photosynthesis in their natural habitat with respect to their optimal photosynthetic temperatures and light saturation points, compared to their potted counterparts. The results of the comparison of field and potted plant, light and temperature responses thus suggests that there was a shift in the photosynthetic response of the site 1 potted plants in response to their new growth habitat.

Site 2 field plants unlike site 1 plants, had a higher optimal photosynthetic temperature (35°C) than their potted counterparts (30°C). The field plants also differed to the potted plants, in that they light saturated at 900μmol $m^{-2}.s^{-1}$ compared to the potted plants, which were light saturated at 1500μmol $m^{-2}.s^{-1}$. The field plants were also inhibited at light intensities above 1100μmol $m^{-2}.s^{-1}$. The yearly mean site temperature (23.56±5.20°C) and light intensity (479.06±226.43μmol $m^{-2}.s^{-1}$) of site 2 was however, different from the optimal field photosynthetic temperature and light saturation point.

The site 3 field optimal photosynthetic temperature (30°C), was similar to site 2, which was also different from their potted counterparts (35°C). The field plants were light saturated at double the light saturation point of their potted counterparts (1500μmol $m^{-2}.s^{-1}$ and 750μmol $m^{-2}.s^{-1}$ respectively). The field plants optimal photosynthetic temperature was also different to the field mean yearly temperature (24.75±6.62°C). The light saturation points of the potted plants of site 3 were closer to the mean yearly light intensities (443.31μmol $m^{-2}.s^{-1}$), similar to site 2. The light and temperature responses for the field

and potted plants were thus not comparable. Thus suggesting that site 3 was similar to sites 1 and 2, in that there was a difference between the gas exchange characteristics of the field and potted plants, which may have been attributed to the new growth environment of the glasshouse that the plants were grown in.

6.1.4.3 *The response of potted Mariscus congestus plants from sites 1-3 to differing CO_2 and O_2 concentrations*

The A_{max}, compensation points, and stomatal limitations of sites 1-3 were not inhibited by differing oxygen concentrations, suggesting that the *M. congestus* leaves of the potted plants from all sites were typically C_4 (Furbank & Hatch 1987; Hatch 1987; Edwards *et al.* 1985; Ku & Edwards 1978; Ehleringer & Björkman 1977; Johnson & Brown 1973). The apparent Km and V_{max} of the sites were however, affected by oxygen concentration.

6.1.4.3.1 *The potted plants response of A_{max} to differing oxygen concentrations*

Site 1 potted plants attained the highest A_{max} in response to the different concentrations of oxygen, site 3 lowest and site 2 an intermediate A_{max}. The A_{max} of sites 1 and 2 were not significantly different, suggesting that least at 350µl/l CO_2, 1500µmol $m^{-2}.s^{-1}$ PPFD and their optimal temperatures, the response of these plants to oxygen concentrations were similar.

6.1.4.3.2 *The potted plants CO_2 compensation points*

Site 1 potted plants had the second highest compensation points. The compensation points of sites 1 and 3 were atypically high for plants with the C_4 photosynthetic pathway (Monson *et al.* 1984; Raghavendra & Das 1976; Krenzer *et al.* 1975; Downton & Tregunna 1968). High compensation points are however, not unusual for a few species in the families Cyperaceae and Gramineae (Krenzer *et al.* 1975). Site 1 and 3 potted plants may thus fall within this category for the family Cyperaceae. Site 2 leaves however, did not have high compensation points. Their compensation points were closer to 0µl/l and thus were

typically C_4 (Raghavendra & Das 1976; Krenzer *et al.* 1975). The compensation points for all the sites were however, not significantly different.

6.1.4.3.3 ***Stomatal limitations on photosynthesis for the potted plants at differing oxygen concentrations***

Site 1 and 3 plants had the lowest stomatal limitations on photosynthetic gas exchange, with site 1 attaining the lowest stomatal limitations. Sites 1 and 3 attained negative values for stomatal limitation, site 1 at 16 and 21% and site 3 at 8 and 21%. It is probable that these negative values for the stomatal limitation may have been due to stomatal patchiness. Stomatal patchiness results in a decreased rate of CO_2 assimilation at a constant internal carbon dioxide concentration, but does not necessarily imply that there is an inhibition on the photosynthetic capacity of the leaf. Patchiness may be explained by assuming the stomata close in patches, as opposed to all closing slightly in response to stress. The part of the leaf shut-off by the closed stomata will thus not have any significant gas exchange with the surrounding environment (Von Kraalingen 1990). It is probable that the effect of the above oxygen concentrations on the leaf within the cuvette may have caused the stomata of sites 1 and 3 leaves to close, resulting in the observed negative stomatal limitations. The low stomatal limitations of site 1 and 3 also suggest that the stomatal control on the photosynthesis of these plants at the different oxygen concentrations was minimal. These results also suggest that the stomata in the field may not have had large influence on the control of CO_2 assimilation. Site 2 plants however, had the highest stomatal limitations, which suggests that there was a significant resistance to CO_2 uptake by these plants at the different oxygen concentrations (Farquhar & Sharkey 1982).

6.1.4.3.4 ***The effects of oxygen on the apparent V_{max} and Km of the potted plants***

The approximated values of $^{app}V_{max}$ and ^{app}Km were determined using Lineweaver-Burke plots (Figs. 38, 40 & 42), which are more accurate than values extrapolated from the A/Ci curves shown in Figs. 37, 39 and 41. Higher apparent Km and V_{max} values are attained using the Lineweaver-Burke plots, due to the intercept on the Y axis (1/A) being close to the X-Y axis origin. The Lineweaver-Burke plots shown

reflect both biochemical and structural resistances. The interpolated $^{app}V_{max}$ and ^{app}Km values thus cannot be a true reflection of the V_{max} or Km of Rubisco, which can only be determined using isolated enzymes. The calculated values are nonetheless useful, as they may be used to estimate the apparent effect(s) of manipulable variables such as oxygen partial pressure on whole leaf photosynthesis. Nonetheless, the use of Lineweaver-Burke plots has found favour in the past (Monson *et al.* 1984; Ku & Edwards 1977).

It is therefore not supprising that the calculated $^{app}V_{max}$ and ^{app}Km values (Tables 5 & 6) are higher than the A_{max} values and CO_2 concentrations obtained under laboratory conditions (Figs. 37, 39 & 41). The $^{app}V_{max}$ and ^{app}Km values for *M. congestus* from sites 1-3, are however, similar to those obtained for *T. aestivum* by Ku & Edwards (1977). The $^{app}V_{max}$ and ^{app}Km values (Tables 5 & 6) of site 2 *M. congestus* were the highest, site 1 was intermediate and site 3 the lowest.

The high $^{app}V_{max}$ and ^{app}Km values of site 2 plants, suggest that the limitations imposed by stomata on the CO_2 assimilation rates, were not the only factors that contributed to the relatively low assimilation rates recorded under field and laboratory conditions. The data suggests that there may be biochemical or structural factors involved in the regulation of the measured CO_2 assimilation rates. The regulation of CO_2 assimilation in these plants will need an in depth study to determine which factors are involved in assimilation depression below the interpolated $^{app}V_{max}$ and ^{app}Km values obtained.

The results of experiments conducted on site 1 plants in contrast had high $^{app}V_{max}$ and ^{app}Km values, as well as high A_{max} values, and low stomatal limitations. This data again suggests that site 1 plants may have imposed biochemical and/or structural limitations to CO_2 assimilation. Again further research would be required to determine the factors involved in the regulation of their photosynthetic CO_2 assimilation and is thus beyond the scope of this investigation.

In contrast, site 3 plants were significantly affected by changes in oxygen partial pressure, more especially at 8 and 16%, where the apparent Km values were below ambient CO_2 levels (Table 6). The low ^{app}Km values correlate to the low $^{app}V_{max}$ (Table 5) and A_{max} values (Fig. 41) attained in response to different oxygen concentrations under laboratory conditions as well as overall lower field photosynthetic assimilation rates.

6.2 CONCLUSIONS

The leaves of sites 1-3 were all C_4 and possibly either NADP-ME or PCK. The sites all displayed typical Chlorocyperiod group anatomy and anatomical characteristics typical of the genus *Mariscus*. There was however, a few subtle differences between the coastal (site 1) and the inland (sites 2 and 3) plants. The differences in habitat conditions at the different sites may thus have resulted in the ecotypic divergence of the coastal *Mariscus congestus* plants from the inland plants.

The natural habitats of the sites all had different microclimates. The gas exchange characteristics of *M. congestus* at each site was thus ecotypic, in response to the differing habitat microclimates. Site 1 plants attained the highest CO_2 assimilation, and transpiration rates, stomatal conductance, and water use efficiency in response to its harsh, low soil moisture, high light environment. Site 3 had the lowest CO_2 assimilation, transpiration rates, stomatal conductance, and water use efficiency in response to its low soil moisture, low light environment.

The optimal photosynthetic temperatures and lack of oxygen inhibition on the CO_2 assimilation rates of sites 1-3 potted plants confirms the anatomical studies suggestions that the leaves of these plants were typically C_4. The response of the potted plants to the light, temperature, carbon dioxide and oxygen concentrations suggested that there were many similarities between site gas exchange

characteristics of the potted plants under similar conditions. The only site-specific characteristic that all potted plants displayed, that may have been as direct result of the genotypic adaptations of these plants to their original habitat microclimates, was the point of light saturation. It thus seems that *M. congestus* grown under similar conditions, that originated from differing microclimates and habitats, had similar gas exchange characteristics and displayed few ecotypic characteristics in their new habitat.

The light and temperature response of the site plants in their natural habitats was however, different to the light and temperature response of the potted plants. This may however, have been due to the differing water regimes of the sites and the time of month that the data was recorded. The differences between the potted plants and the field may also have been due to root binding or a reduction in root mass in the pots of the potted plants. The results of this investigation thus suggest that the field and potted plant gas exchange characteristics were not comparable. The results of this investigation thus do not support the hypothesis stated in this thesis that **plants removed from their natural environments would not cause a shift in gas exchange characteristics**. Thus the results of this investigation are at variance with those of Milner and Hiesey (1964), Green (1969) and of Slayter and Ferrar (1977). Their findings were that the physiological response of plants from different environments grown under uniform conditions still displayed ecotypic physiological responses. The results of this investigation thus suggest that even though the anatomy may have undergone ecotypic divergence in response to its original habitats, the physiological characteristics (gas exchange characteristics) had not. The results of this investigation thus support the following alternative hypothesis, that **plants with as diverse environmental ranges such as *Mariscus congestus*, adapt to new habitats rapidly, with little physiological evidence of their previous habitat remaining under new environmental conditions.**

6.3 ACKNOWLEDGEMENTS

I would like to thank the following for their funding, assistance and use of facilities.

1) The Foundation for Research and Development for the award of a research studentship.

2) Rhodes University Botany Department for a Graduate Assistants bursary, without which this investigation would not have been possible.

3) Prof C.E.J. Botha for making the facilities available, for his invaluable assistance, guidance, encouragement and precious time, is gratefully acknowledged.

4) Miss C.Cameron and Prof R.Evert for their kind interest and help.

5) Miss A.Sinclair for her kind help in proof reading this thesis.

6) I also acknowledge the municipalities of Kenton-on-sea and Grahamstown, as well as Prof R.Lubke for the use of their land for this investigation.

7) Finally my parents, because without their help and assistance this thesis would never have been possible.

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APPENDIX 1

Field average daily gas exchange data for sites 1-3, measured from April 1990 to March 1991

APPENDIX 1

The tables 1-8 represent the average daily gas exchange and climatic data for sites 1-3, measured monthly from April 1990 to March 1991. The data in tables 1-8, is also the raw data used to construct Figs. 23-31.

Table 1. The average daily light intensity of sites 1, 2 and 3, investigated monthly from April 1990 to March 1991.

Month	Average monthly light intensity ($\mu mol\ m^{-2}.s^{-1}$)		
	Site 1	Site 2	Site 3
April 1990	310.44	360.00	350.96
May 1990	996.96	360.83	260.90
June 1990	756.43	381.44	210.91
July 1990	916.43	217.61	357.34
August 1990	715.35	-	644.14
September 1990	976.49	751.26	-
October 1990	1308.33	653.70	669.71
November 1990	1214.39	-	588.74
December 1990	1189.26	-	148.54
January 1991	1183.35	-	-
February 1991	1136.78	292.04	758.51
March 1991	979.94	815.62	-

Table 2. The monthly percentage soil moisture of sites 1, 2 and 3, investigated from April 1990 to March 1991.

Month	Average monthly percentage soil moisture (%)		
	Site 1	Site 2	Site 3
April 1990	4.13	20.40	4.51
May 1990	2.93	21.31	8.08
June 1990	2.04	18.50	5.94
July 1990	0.80	12.59	7.59
August 1990	2.17	16.10	5.77
September 1990	1.12	16.05	10.95
October 1990	2.40	16.60	10.55
November 1990	2.17	5.86	2.35
December 1990	0.23	3.73	2.11
January 1991	1.59	4.41	2.33
February 1991	1.73	8.60	2.60
March 1991	5.00	5.27	3.19

Table 3. The average daily temperatures of sites 1, 2 and 3, investigated monthly from April 1990 to March 1991.

Month	Average monthly temperature (°C)		
	Site 1	Site 2	Site 3
April 1990	18.05	25.15	23.84
May 1990	25.46	17.32	12.87
June 1990	22.45	20.71	17.28
July 1990	23.95	13.50	22.72
August 1990	20.81	26.70	26.76
September 1990	29.03	23.60	23.61
October 1990	26.69	24.22	23.87
November 1990	29.23	26.20	26.17
December 1990	33.09	20.70	20.86
January 1991	32.22	31.00	30.00
February 1991	30.15	22.06	38.97
March 1991	29.57	31.50	30.00

Table 4. The average daily CO_2 assimilation rates of sites 1, 2 and 3, recorded monthly from April 1990 to March 1991.

Month	Average monthly CO_2 assimilation rates (µmol $m^{-2}.s^{-1}$)		
	Site 1	Site 2	Site 3
April 1990	9.20	2.27	2.68
May 1990	11.20	2.26	0.34
June 1990	9.40	4.21	0.17
July 1990	13.38	3.76	2.11
August 1990	18.18	-	4.23
September 1990	23.96	11.60	-
October 1990	23.87	6.88	5.59
November 1990	14.64	-	3.93
December 1990	18.60	-	1.78
January 1991	23.63	-	-
February 1991	27.75	4.91	0.40
March 1991	27.25	3.02	-

Table 5. The average daily transpiration rates of site 1, 2 and 3 leaves, investigated monthly from April 1990 to March 1991.

Month	Average monthly transpiration rates (mmol $m^{-2}.s^{-1}$)		
	Site 1	Site 2	Site 3
April 1990	3.66	2.86	1.66
May 1990	4.89	2.61	0.36
June 1990	3.72	10.49	2.60
July 1990	10.58	3.24	2.09
August 1990	6.19	-	4.65
September 1990	14.11	7.01	-
October 1990	12.69	4.39	6.12
November 1990	7.91	-	7.20
December 1990	8.61	-	6.73
January 1991	14.47	-	-
February 1991	12.67	3.04	3.74
March 1991	16.64	2.26	-

Table 6. The average daily water use efficiency of sites 1, 2 and 3, investigated monthly from April 1990 to March 1991.

Month	Average monthly water use efficiency ($1x10^{-3}mol.mol^{-1}$)		
	Site 1	Site 2	Site 3
April 1990	3.00	0.58	1.70
May 1990	4.00	1.71	0.41
June 1990	3.00	0.24	0.20
July 1990	1.00	1.05	0.95
August 1990	2.00	-	0.97
September 1990	2.00	1.65	-
October 1990	2.00	1.57	0.93
November 1990	2.00	-	0.56
December 1990	2.00	-	0.29
January 1991	2.00	-	-
February 1991	2.00	2.09	0.20
March 1991	2.00	1.41	-

Table 7. The average daily stomatal conductance of sites 1, 2 and 3 leaves, investigated monthly from April 1990 to March 1991.

Month	Average monthly stomatal conductance rates ($mmol\ m^{-2}.s^{-1}$)		
	Site 1	Site 2	Site 3
April 1990	241.62	107.17	405.61
May 1990	94.87	141.89	130.20
June 1990	127.23	945.69	159.70
July 1990	664.26	274.79	81.54
August 1990	358.17	-	161.93
September 1990	684.80	348.67	-
October 1990	540.00	177.06	294.73
November 1990	265.76	-	398.47
December 1990	247.52	-	412.22
January 1991	545.15	-	-
February 1991	519.12	301.67	61.06
March 1991	916.14	54.14	-