Research paper

Using Vector Analysis to Assess the Nutrient Status of *Lumnitzera racemosa* Seedlings under Different Salinities

Kuei-Chu Fan[1] Bor-Hung Sheu[2]

【**Abstract**】Mangrove seedlings *Lumnitzera racemosa* were grown in different concentrations of salinity containing 0, 0.75, 1.5, 3.0, 4.5 and 6.0% NaCl for periods of 60, 90 and 120 days. The variable trends of three harvest periods were markedly similar, therefore only the data for the 120 days harvest were presented. The application of vector analysis to assess nutrient status of seedlings under different salinities was studied. Seedling growth was significantly stimulated in 0.75% NaCl treatment, but inhibited in 1.5% NaCl treatment, although the leaves appeared both green and healthy. Diagnosis results indicated that seedlings grown in 0.75 and 1.5% NaCl displayed a significant deficiency for osmoregulation required Na and Cl ions. The K, N, Mg and Ca nutrients in the leaves displayed dilution effects that did not inhibit the growth of seedlings for both salinity treatments. Seedlings grown in a concentration of 3.0% NaCl were significantly suppressed, and seedlings grown in concentrations of 4.5 and 6.0% NaCl died by the end of the experiment. Diagnostic results confirmed that seedlings grown in hypersaline conditions of more than 3.0% NaCl salinity expressed an excessive uptake of Na and Cl ions, resulting in a toxicity for the seedlings. The N, Mg, K and Ca ions in the leaves also displayed antagonistic effects that due to an excess accumulation of Na and Cl ions.

【**Key words**】*Lumnitzera racemosa*, Nutrient status, Mangrove, Salinity, Vector analysis.

研究報告

利用向量分析評估欖李苗木在不同土壤鹽度下之養分狀態

范貴珠[1] 許博行[2]

【**摘要**】紅樹林樹種欖李（*Lumnitzera racemosa*）苗木以 0、0.75、1.5、3.0、4.5 及 6.0 % NaCl 等不同鹽度溶液，分別處理 60、90 及 120 天。而此三個取樣時期的苗木生長情形相似，因此僅以第 120 天的資料，利用向量分析（vector analysis）評估不同土壤鹽度對苗木養分狀態之影響。結果顯

1. Associate Professor, Department of Forestry, National Pingtung University of Science and Technology. Corresponding author.
國立屏東科技大學森林系副教授，通訊作者
2. Professors, Department of Forestry, NCHU.
國立中興大學森林學系教授

示欖李苗木在 0.75 % 鹽度中生長最佳，1.5 % 鹽度處理之葉片雖呈濃綠健康狀態，但苗木生長已受抑制。而診斷分析結果顯示，生長於 0.75 及 1.5 % 鹽度之苗木，其葉片 Na 及 Cl 離子濃度明顯缺乏，仍不足以供滲透調節作用進行。葉片 K、N、Mg 及 Ca 則呈現生長稀釋之影響，因此不會影響此二種鹽度對苗木之生長。至於 3.0 % 鹽度已明顯抑制苗木生長，4.5 及 6.0 % 處理之苗木則在處理後期逐漸死亡。而診斷分析結果證實，苗木若生長在超過 3.0 % 以上之高鹽分環境時，苗木會過量吸收 Na 及 Cl 離子導致毒害；而且葉片之 N、Mg、K 及 Ca 離子濃度，則因 Na 及 Cl 離子過量累積而呈現拮抗作用。

【關鍵字】欖李、養分狀態、紅樹林、鹽度、向量分析

I. Introduction

Mangrove vegetation is located in both tropical and subtropical intertidal zones and is dominated by halophytic woody trees and shrubs (Tomlinson, 1994). In the case of a mangrove forest, nutrient limitation, waterlogging and soil salinity are hypothesized as the principal factors that control mangrove growth (Naidoo, 1985; Ball, 1996; Koch, 1997; Koch and Snedaker, 1997). In particular, mangrove environments are characterized by large variations in salinity (both spatially and seasonally), and are related to regional climates and topographic features (Duke, 1992; Tomlinson, 1994). Numerous researchers have confirmed that soil salinity is an important factor associated with mangrove growth, and that diverse mangrove species have different tolerances and optimal salinity (Ball, 1988; Hwang and Chen, 1995; Smith and Snedaker, 1995; Koch, 1997).

The mechanisms by which salinity inhibits mangrove growth have been attributed to many factors including: (1) a decrease in the water potential of soil and plant tissue (Naidoo, 1985; 1987; Ball, 1988; Suárez *et al.*, 1998), (2) a decline in the rate of photosynthesis (Ball and Anderson, 1986; Werner and Stelzer, 1990; Lin and Sternberg, 1992), and (3) an excessive uptake of salts that interfere with physiological metabolism (Mizrachi *et al.*, 1980; Burchett *et al.*, 1984; Fan *et al.*, 1999) or cause nutrient imbalance (Downton, 1982; Clough, 1984; Hwang and Chen, 1995; Koch and Snedaker, 1997). The presence of salt ions alters the nutritional balance of plants by one or more mechanisms, including affecting nutrient availability, competitive interactions among ions in the substrate, affecting osmotic potential and membrane selectivity (Kozlowski, 1997; Ruiz *et al.*, 1997; Grattan and Grieve, 1999). Further research regarding the complex effects of salinity on plant nutrients is required to determine both the content and nutrient elements of seedlings in different salinities, and how a change in salinity related to plant growth.

Vector analysis provides an effective means for assessing the extent to which controlled treatment effects are linked to nutrient supply (Timmer and Armstrong, 1987; Timmer, 1991; Haase and Rose, 1995). By plotting the relative changes in nutrient concentration, the content and foliage biomass on the nomogram, the extent to which different nutrients limited tree growth can be evaluated. Vector analysis has been extensively employed to evaluate the fertilizer responses and numerous combinations of stress in conifers

(Timmer and Armstrong, 1987; Imo and Timmer, 1997; Kiefer and Fenn, 1997). This diagnostic tool has also been employed to improve interpretations of several aspects in the silviculture (Macdonald *et al*., 1998; Imo and Timmer, 1999; Proe *et al*., 1999) and the effects of pollution (Weetman *et al*., 1993; Brække, 1996).

Black mangrove (*Lumnitzera racemosa*) is a non-viviparous and no prominent aerial roots angiosperm, commonly located at the landward interface of intertidal zones in Eastern Africa to the Western Pacific, tropical Australia and Indochina (Tomlinson, 1994). The population of *L. racemosa* in Taiwan has substantially declined as a result of industrial and agricultural development. If this trend continues, this species will disappear (Huang *et al*., 1998). In the open areas, the tree retains its rounded, low-crowned shape, and its white flowers are visited by a variety of day-active wasps, bees, butterflies and moths. Therefore, this mangrove species is recommended as a proper greenery species on the salt marshes and coastal areas of Taiwan. Only a few studies have focused on the salinity responses of this species. This study investigated how different salinities affected seedling growth, and the vector analysis was applied to assess the complex nutrient status of seedlings cultivated under different salinities.

II. Materials and methods

(I) Plant culture and treatments

Mature fruits of *L. racemosa* were collected in the Ssu-tsa area, Tainan City (23°00' N, and 120°05'E), Taiwan, in July 1997. After germination in vermiculite, the seedlings were transferred to plastic pots (15 cm in diameter, 1.3 L in volume) and filled with sand, peat and vermiculite (2:1:2 in volume). Twelve pots were placed in a plastic tray (60 cm length × 48 cm width × 16 cm height) and submerged in Hoagland's solution (Hoagland and Arnon, 1950; Johnson *et al*., 1957) containing 0, 0.75, 1.5, 3.0, 4.5 and 6.0% NaCl, respectively. There were four replicates per treatment, and each replicate contained 36 seedlings. The level of the culture solution in the tray was maintained each day, and these solutions were replaced with a fresh solution every 30 days. Plants were grown in a naturally illuminated glasshouse with approximately 820-1200 $mmol\,m^{-2}s^{-1}$ photo-synthetic photon flux density (PPFD). The air temperature for the experimental period was approximately 25-30℃.

(II) Growth measurement

Seedlings were harvested to investigate the morphological growth and analyzed nutrient elements in leaves after a period of 60, 90 and 120 days. Twelve seedlings of each treatment were harvested and measured individually for height, root collar diameter and leaf area (LI-3000A, LI-COR). The seedlings were rinsed carefully with distilled water, and oven dried at 70℃ for one week.

Leaf succulence (water per unit leaf area) was determined on three to four fully expanded leaves of a similar age. Twenty leaf disks were excited at 10 to 12 a.m. and placed in vials with 5 ml distilled water to re-hydrate in the dark for 24 hr. The following day, the leaf turgid weight (TW) was measured and dried at 70℃ for 48 hr to determine the dry weight (DW). The degree of succulence was calculated by the equation (Gucci *et al*., 1997).

water content at saturation (mg) = leaf turgid weight (TW) − dry weight (DW)

degree of succulence (mg H_2O/cm^2) = water content at saturation (mg)/surface area (cm^2)

(III) Foliage sampling and nutrient analysis for vector diagnosis

Twenty full-expanded mature leaves were randomly sampled from three seedlings of each treatment with three replicates. Samples were oven dried at 70℃ for one week to determine the dry weight of 20 leaves. Total N of the dried leaves was analyzed with a sulphuric acid-copper sulphate digestion method. The leaves were digested in a concentrated nitric: perchloric: sulphuric acid (10:4:1, v/v) mixture, and then P was measured by the molybdenum-blue method; Na and K were measured by flame photometry; Ca and Mg were measured by atomic absorption spectrophotometry (Kalra and Maynard, 1991). Chloride was extracted from 0.2 g of leaves with 5 ml of deionized water (Ghosh and Drew, 1991) and measured by silver ion titration with a Jenway chloridometer (Jenway, Model PCLM3, Essex, England).

(IV) Vector diagnostic analysis

For construction and interpretation of nomograms, the nutrient concentration, nutrient content and dry weight are plotted as vector according to the technique described by Timmer and Armstrong (1987), Haase and Rose (1995). A vector graph consists of the foliar element content on the x-axis, the foliar element concentration on the y-axis, and the foliage dry weight on the z-axis. The foliage dry weight (z-axis) is represented by a series of diagonal lines of equal dry weight (Fig. 1). All values have

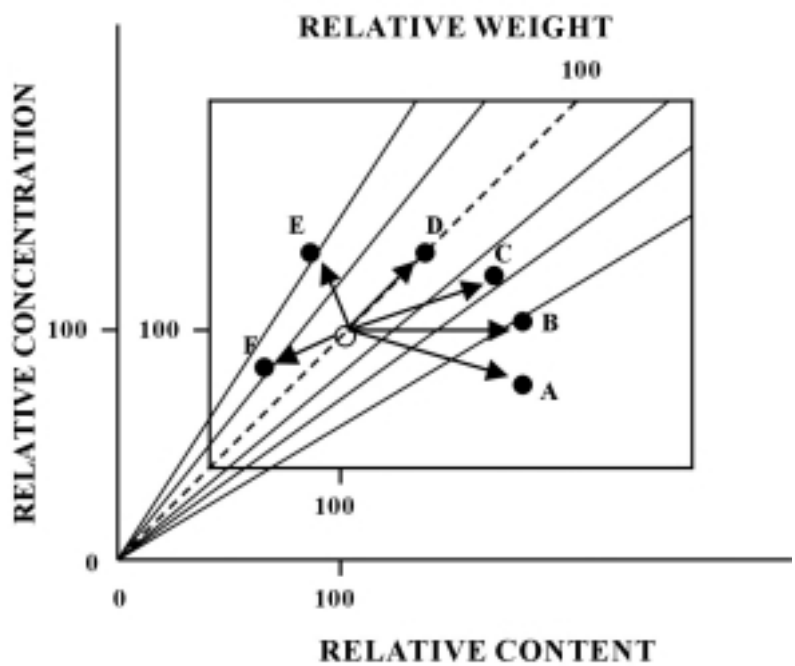

Fig. 1. Directional shifts in the nutrient concentration, nutrient content and dry weight. Adapted from Timmer and Armstron (1987).

been standardized to a value of 100 for seedlings grown in 0% NaCl as a control treatment. For each salinity and nutrient, the average value of the concentration and content from treated seedlings was calculated in relation to the average value of the control seedlings. The direction and magnitude of the vector in each nutrient connecting the control and treated values was used to diagnose the nutritional status of seedlings and their response to different salinities.

Table 1 showed the interpretation of directional shifts from fig. 1. A shift towards A signifies decreasing concentration but increasing leaf weight and content; hence the supply of the supply of the nutrient has been diluted by additional growth, suggesting that the specific nutrient is not the major limiting nutrient. An outward horizontal shift B, whereby weight and content increase without change in concentration. This horizontal shift may be as "minimum percentage" range resulting from nutrient transport into the foliage just sufficient to keep pace with shoot or leaf expansion or redistribution within the plant. A shift towards C denotes increases in both nutrient and concentration and leaf weight. This implies that the initial level was limiting, and the sector between radii B and D would correspond to "poverty adjustment" range. Movement towards D, resulting from increased accumulation without any gain in leaf weight, may be interpreted as luxury consumption by the foliage. Movement in the direction of E results from concentration increase combined with reduced leaf weight. This is a strong indication of toxic accumulation, unless associated with some other growth constraint (Timmer and Stone, 1978).

(V) Statistical analyses

The ANOVAs were calculated for each harvest based on six treatments and three replicates per treatment per seedling with the STATGRAPHICS package (Manugistics, Inc).

III. Results

(I) Growth

After salinity treatment for 120 days, the overall morphological parameters including height, diameter at root collar, total leaf area and dry matter accumulation of seedlings was showed

Table 1. Interpretation of the directional shifts from Fig. 1.

Direction	Change in relative			Interpretation	Possible diagnosis
	Leaf	Nutrient			
	weight	Conc.	Content		
A	+	–	+	Dilution	Non-limiting
B	+	0	+	Sufficiency	Non-limiting
C	+	+	+	Deficiency	Limiting
D	0	+	+	Luxury consumption	Non-toxic
E	–	+	±	Excess	Toxic accum
F	–	–	–	Excess	Antagonism

+: increase; 0: no change; –: decrease; ±: increase or decrease

in Table 2. Seedlings cultivated in 0.75% NaCl displayed the most significant enhance. The mean total dry weight of the seedlings was 10.4 g. All other parameters and the biomass of the seedlings gradually following the NaCl level increased or decreased. There was a sharp and significant decrease in the seedlings grown in 3.0% NaCl, the total biomass was only 3.4g which was approximately the total biomass of 1/3 of the seedlings cultivated in 0.75% salinity. In the hypersaline conditions of the 4.5% and 6.0% NaCl treatments, and the growth of seedlings were almost deadlocked, the total dry weights of the seedlings were 0.6g and 0.4g, respectively.

(II) Nutrient status

After the seedlings were cultivated for 120 days under different salinities, the average dry weights of 20 leaves were from 0.52g to 1.30g (Table 3). The seedlings cultivated in 0.75% and 1.5% NaCl displayed a higher dry weight of leaves than in other treatments. The tissue concentrations of Na and Cl in the leaves increased with an increase in salinity. The concentrations of N, K, Ca and Mg in the leaves decreased with an increase in salinity, and no change in the P concentration was observed. The content of each nutrient was calculated for each treatment by multipling the nutrient concentration (y) by unit dry weight (z). The data for the unit dry weight, nutrient concentration and content of the seedlings grown in 0% salinity were normalized to 100, and were taken as a reference for comparison with the seedlings grown in 0.75, 1.5, 3.0, 4.0 and 6.0% treatments (values of parenthesis in Table 3). The data were illustrated by using vector nomograms in Figures 2-5.

(III) Vector diagrams and interpretation

The relative leaf weight of the seedlings treated with 0.75% NaCl was 112 in comparison with the control treatment (Table 3). A vector analysis of the Cl and Na contents of the foliage displayed a particularly long magnitude of C-shift (i.e., deficiency as limiting elements) (see Figures 1 and 2A). The P content also displayed a C-shift, but the relative concentration of P increased from 100 in control seedlings to only 103 (Table 3). The magnitudes were shorter and closer to the control treatment (Fig. 2B). A vector analysis of the K, N, Mg and Ca contents displayed A-shifts (i.e., dilution as a non-limiting element) (see Figures 1

Table 2. Growth parameters of seedlings cultivated in different salinities for 120 days[1)].

	Salinity					
Parameter	0%	0.75%	1.5%	3.0%	4.5%	6.0%
Height (cm)	27.0 ± 1.7^{b}	33.9 ± 1.3^{a}	26.3 ± 1.7^{b}	18.4 ± 1.3^{c}	9.8 ± 0.9^{d}	8.8 ± 0.7^{d}
Diameter (mm)	6.4 ± 0.1^{a}	7.1 ± 0.3^{a}	6.4 ± 0.2^{a}	5.1 ± 0.2^{b}	2.8 ± 0.1^{c}	2.6 ± 0.2^{c}
Total leaf area (cm^2/per seedling)	454.8 ± 32.0^{b}	666.8 ± 30.7^{a}	433.1 ± 28.4^{b}	166.4 ± 15.8^{c}	19.1 ± 2.4^{d}	8.4 ± 1.5^{d}
Degree of succulence (mg H_2O/ cm^2)	47 ± 1^{c}	58 ± 2^{bc}	64 ± 3^{bc}	70 ± 3^{b}	111 ± 7^{a}	113 ± 7^{a}
Total dry weight (g)	7.0 ± 0.2^{b}	10.4 ± 0.4^{a}	7.3 ± 0.3^{b}	3.4 ± 0.2^{c}	0.6 ± 0.1^{d}	0.4 ± 0.1^{e}

1) Means within a row followed by the same letter are not significantly different at $P < 0.05$, according to the Duncan's test.

Table 3. Leaf dry weight, nutrient concentration and nutrient content of seedlings in different salinities for 120 days. Values in parenthesis are presented as the relative leaf dry weight, nutrient concentration and nutrient content of the seedlings. All values of the seedlings in 0% NaCl were normalized to 100.

Salinity	Dry weight of 20 leaves (g)	Nutrient													
		Concentration of 20 leaves (mg/g)							Content of 20 leaves (mg)						
		N	P	K	Na	Cl	Ca	Mg	N	P	K	Na	Cl	Ca	Mg
0%	1.15	25.8	0.9	14.2	11.8	20.8	11.4	8.0	29.7	1.1	16.3	13.6	24.0	13.2	9.2
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
0.75%	1.30	24.2	1.0	13.8	25.6	50.9	9.0	7.1	31.4	1.2	17.8	33.2	65.9	11.6	9.3
	(112)	(94)	(103)	(97)	(217)	(244)	(78)	(90)	(106)	(116)	(109)	(244)	(275)	(88)	(101)
1.5%	1.26	24.0	0.9	10.6	36.3	56.2	8.0	6.2	30.3	1.2	13.4	45.8	71.0	10.0	7.8
	(109)	(93)	(101)	(75)	(308)	(270)	(70)	(78)	(102)	(111)	(82)	(337)	(296)	(76)	(85)
3.0%	0.84	22.4	1.0	9.4	46.6	64.7	6.0	5.3	18.7	0.8	7.8	39.0	54.1	5.0	4.5
	(73)	(87)	(105)	(66)	(395)	(311)	(52)	(67)	(63)	(76)	(48)	(286)	(225)	(38)	(49)
4.5%	0.53	18.3	0.9	7.1	62.9	92.4	3.6	6.4	9.6	0.5	3.7	33.1	48.6	1.9	3.4
	(46)	(71)	(96)	(50	(533)	(444)	(31)	(80)	(32)	(44)	(23)	(243)	(203)	(14)	(37)
6.0%	0.52	18.5	0.9	7.3	65.0	92.7	3.2	6.4	9.7	0.5	3.8	34.1	48.6	1.7	3.4
	(45)	(72)	(96)	(52)	(551)	(445)	(28)	(81)	(33)	(43)	(24)	(251)	(202)	(13)	(37)

and 2B). The magnitudes of these nutrients were also shorter and closer to the control set.

The variable trends of the leaf dry weight and vector analysis of the seedlings grown in 1.5% salinity were similar to those in 0.75% treatment. The average weight of the leaves was 109 in comparison with the control treatment (Table 3). A vector analysis of the Na, Cl and P in the leaves displayed a C-shift, particularly, the relative contents of Na and Cl increased to 308 and 270, respectively, indicated a more deficient shift than seedlings grown in 0.75% salinity (Fig. 3A). A vector analysis of the N, Mg, K and Ca nutrients displayed A-shifts in this salinity (Fig. 3B).

When the cultivated salinity was increased to 3.0% NaCl, the relative leaf dry weight of the seedlings decreased to 73 in comparison with the 100 of control treatment. Treatment conducted at this salinity resulted in the relative concentrations of Na and Cl increasing from 100 in the control seedlings to 395 and 311, respectively. The relative contents of Na and Cl increased to 286 and 225, respectively (Table 3). A vector analysis of these elements displayed E-shift, (i.e. the direction and magnitude indicated an excessive and toxic accumulation of these elements) (see Figures 1 and 4A). The P in the leaves also displayed E-shift, however, the magnitudes were closer to the control treatment (Fig. 4B). The relative concentrations of N, Mg, K and Ca in the

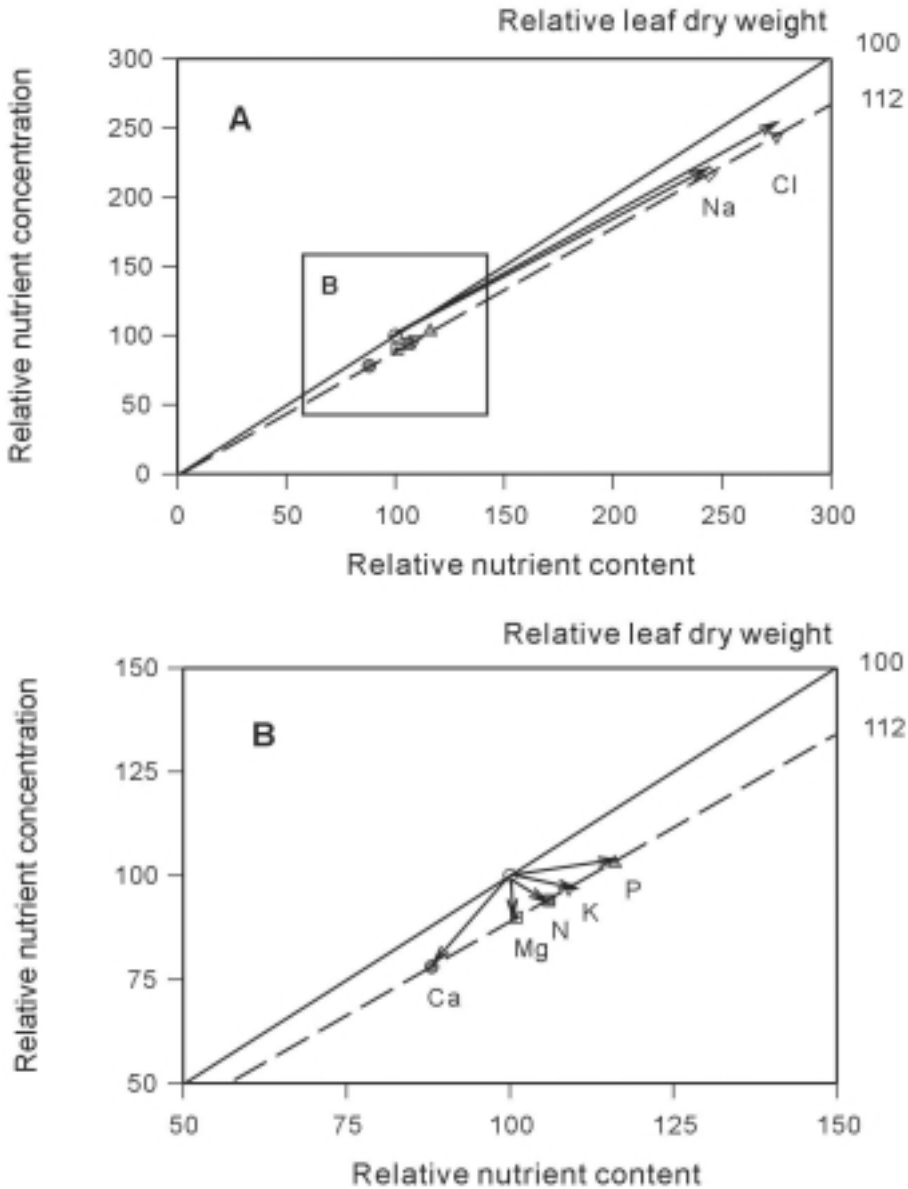

Fig. 2. Vector nomograms of relative changes in the dry weight, nutrient concentration and nutrient content of the seedlings grown in 0.75% NaCl treatment. Nutrient status of seedling in 0% NaCl was normalized to 100.

leaves decreased to 87, 67, 66 and 52, respectively in the treatment of 3.0% NaCl (Table 3). A vector analysis of these elements displayed F-shifts, (i.e. expressed antagonistic effects for seedling growth) (see Figures 1 and 4B).

Seedlings grown in 4.5% and 6.0% hypersaline treatments were almost deadlocked. The leaf dry weight of the seedlings grown in

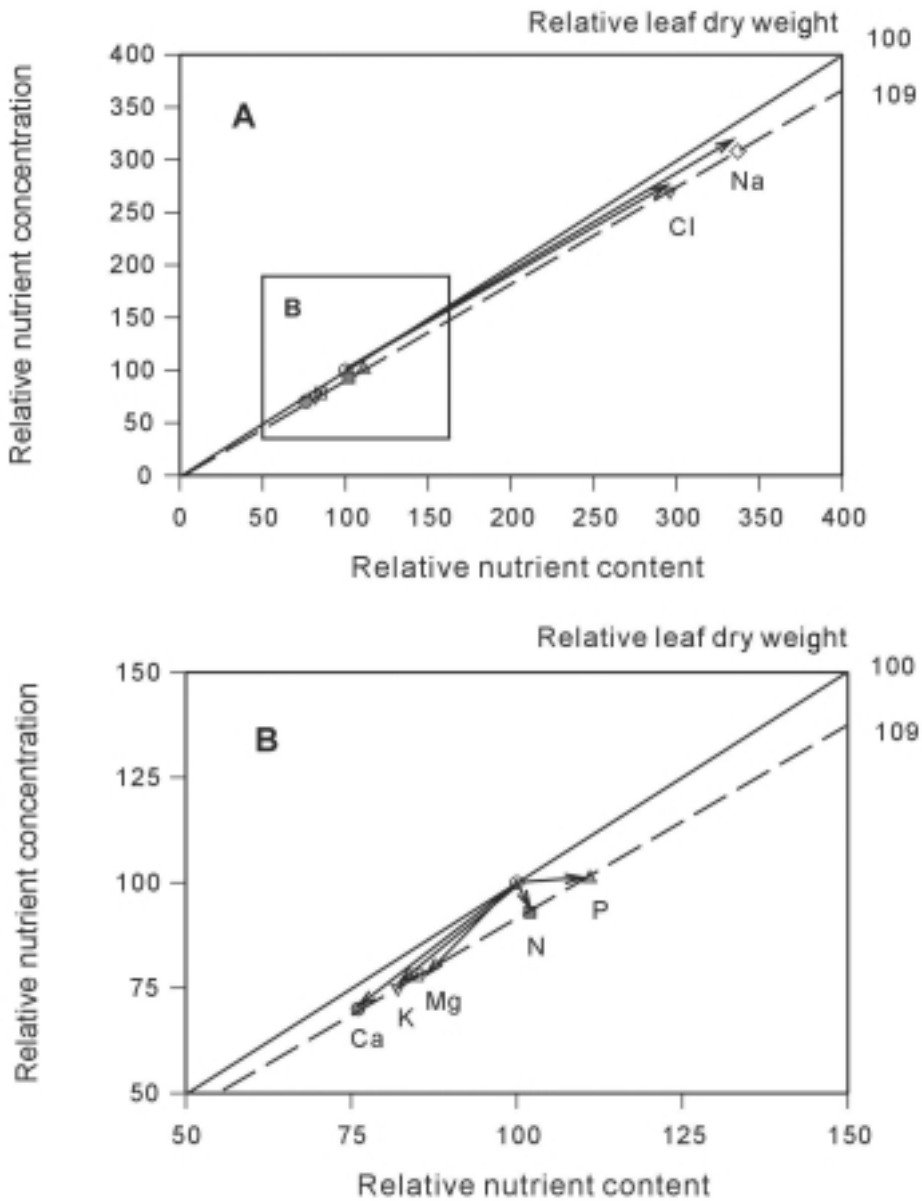

Fig. 3. Vector nomograms of the relative changes in the dry weight, nutrient concentration and nutrient content of seedlings grown in 1.5% NaCl treatment.

4.5% NaCl decreased to 46 in comparison with the 100 of control treatment (Table 3). The Na, Cl and P in the leaves showed a E-shift, implying a severely excessive and toxic accumulation (Fig. 5A). The Mg, N, K and Ca displayed excessive as antagonistic effects (i.e., F-shifts in Fig. 1) (Fig. 5B). The variable trends of the leaf dry weight and vector analysis of seedlings grown in 6.0% were close to the results obtained for the 4.5% treatment (data not shown).

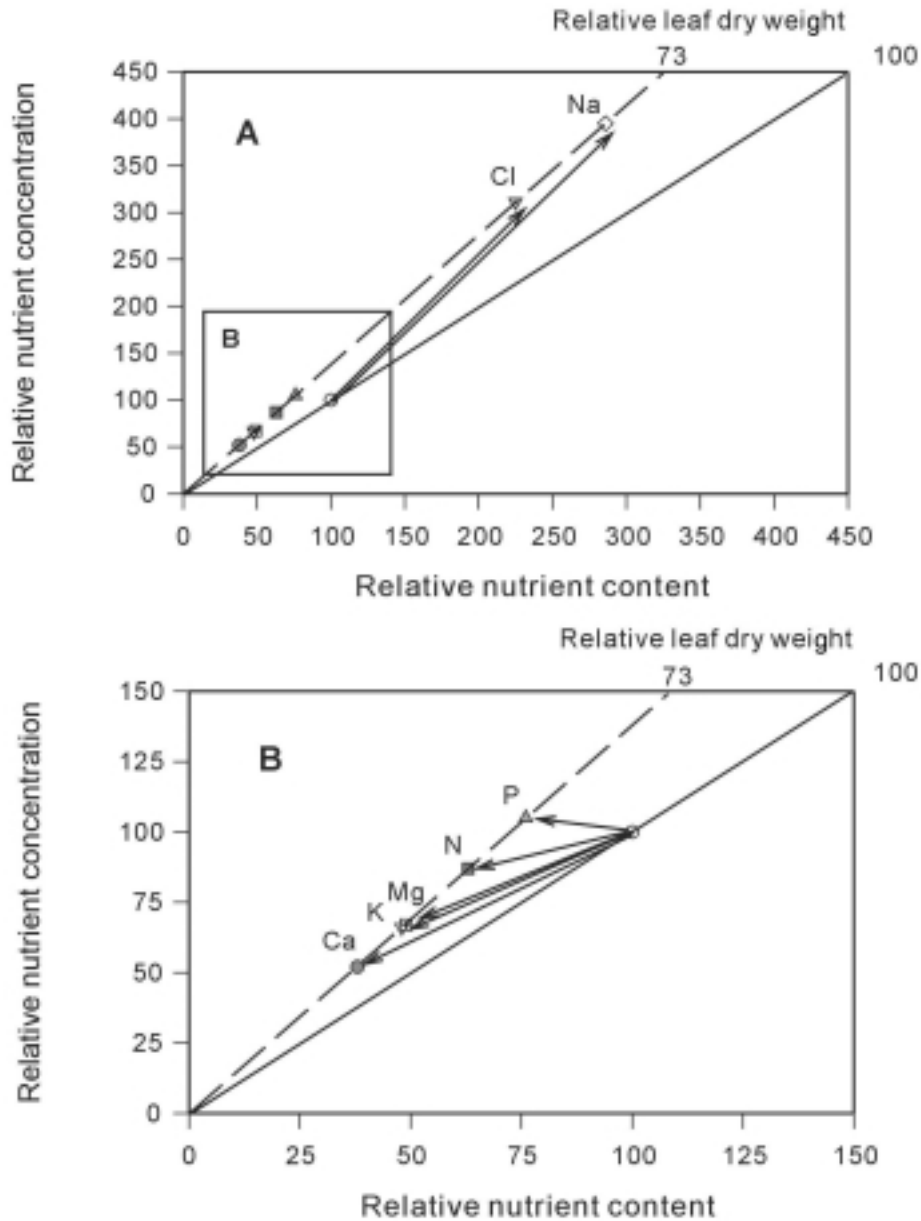

Fig. 4. Vector nomograms of the relative changes in the dry weight, nutrient concentration and nutrient content of seedlings grown in 3.0% NaCl treatment.

IV. Discussion

After 120 days treatments with different salinity media, the growth of *L*. racemosa seedlings decreased as compared to the control treatment without NaCl (Table 2). There was a decline in the chlorophyll concentration and a yellowing of seedling leaves treated with NaCl (Fan, 2000). Such a response of the mangrove species is often attributed to the inability of the plant to accumulate sufficient inorganic ions for

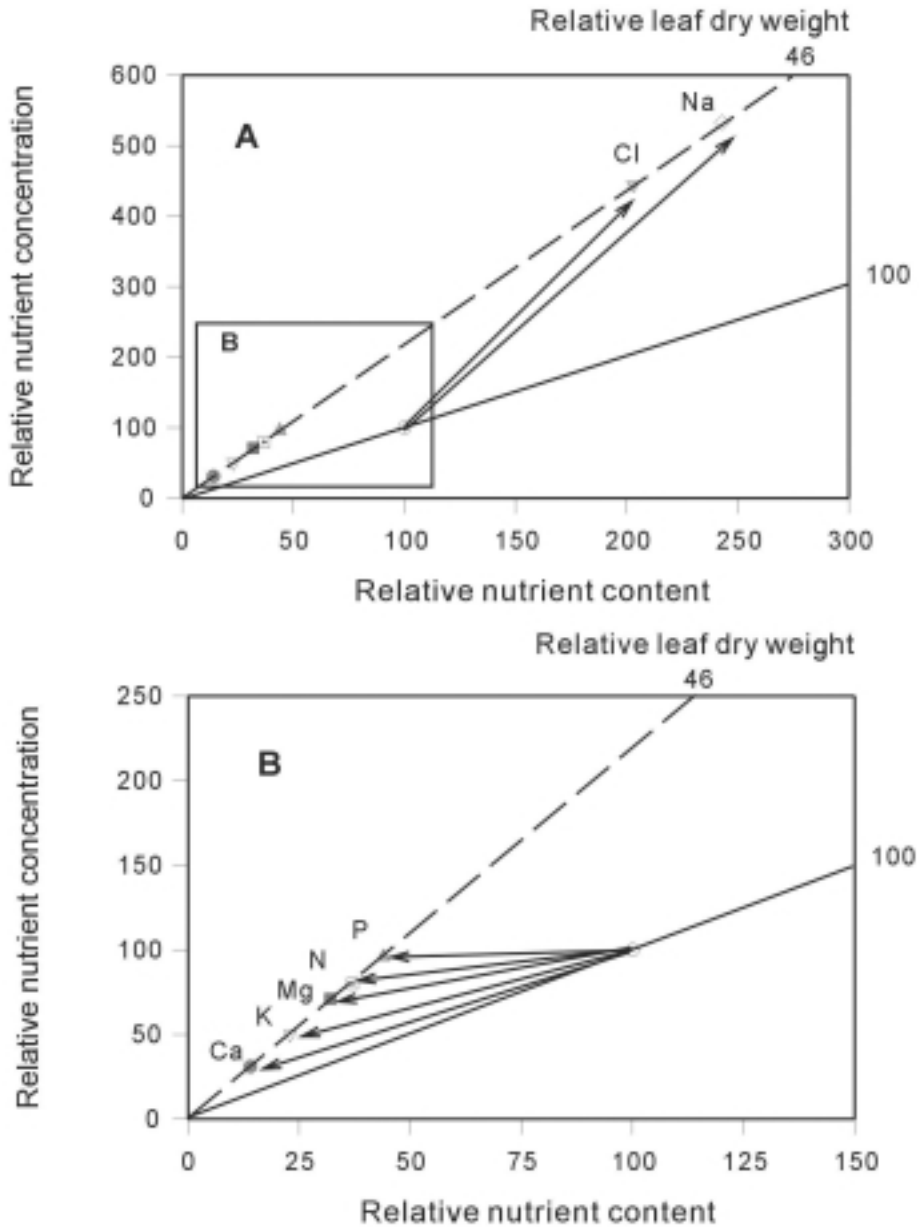

Fig. 5. Vector nomograms of the relative changes in the dry weight, nutrient concentration and nutrient content of seedlings grown in 4.5 % NaCl treatment.

osmoregulation when NaCl is absent in the substrate. This hypothesis is consistent with the effects of water deficiency on cell division and cell expansion (Downton, 1982; Clough, 1984; Hwang and Chen, 1995). Although *L. racemosa* seedlings accumulated high concentrations of K, Ca and Mg in the leaves even when cultured in 0% NaCl treatment (Table 3), it appears that the concentrations of these cations displayed insufficient quantities for osmoregulation,

resulting in a decrease in the growth of seedlings.

According to the results of vector analysis, the average leaf weight of seedlings grown in 0.75% NaCl was 112 in comparison with the control treatment (Table 3). However, the Na and Cl ions (major vector) displayed a larger C-shift magnitude (Fig. 2A) indicating that Na and Cl ions were deficient for seedling growth. Generally, salinity often interferes with the nutritional balance and causes a deficiency of essential elements which limit the growth of plants (Kozlowski, 1997; Koch and Snedaker, 1997; Ruiz *et al*., 1997). However, *L. racemosa* seedlings cultivated in 0.75% NaCl treatment exhibited the greatest height, ground diameter, total leaf area and biomass accumulation (Table 2) and deficient symptoms were not observed. Mallery and Teas (1984), Werner and Stelzer (1990), Hwang and Chen (1995) indicated that Na and Cl play a major biophysical role for the osmotic adjustment of mangrove species. In this study the vector analysis confirmed that *L. racemosa* seedlings grown in 0.75% NaCl required a greater quantity of Cl and Na ions to generate an increase in the negative osmotic potentials than that of the culture medium. Alarcón *et al*. (1993) reported that at lowest salinity, the osmotic adjustment in plants was almost exclusively on Cl and Na ions for a marked energy saving. These findings suggest a positive growth rate for *L. racemosa* seedlings under 0.75% mild salinity.

The vector analysis of *L. racemosa* seedlings cultivated in 0.75% NaCl treatment also displayed the content of P increased and presented as deficient elements (Fig. 2B). The magnitude was closer to the control treatment, indicating that there is less limited effect on the seedlings. However, the relative concentrations of K, N, Mg and Ca were lower than those if the control treatment, and the nomograms of vector diagnosis interpreted the dilution effects as non-limiting nutrients in this mild salinity (Fig. 2B). These dilution effects occurred not only because the leaf growth rate was much higher than nutrient uptake rate, but also attributed to increase of leaf succulence in seedling (Table 2). This process maintained a reasonable constant of salt concentration. This is one of the most important mechanisms for the salt balance in non-secreting mangrove species (Popp *et al*., 1993; Tomlinson, 1994). Therefore, *L. racemosa* similar to the most mangrove species such as *Avicennia marina* (Burchett *et al*., 1984; Clought, 1984; Naidoo, 1987), *Aegiceras corniculatum* (Ball, 1988), *Rhizophora mangle* (Smith and Snedaker, 1995) and *Kandelia candel* (Hwang and Chen, 1995), can grow in water with a salinity ranging between 10 to 25% of seawater.

When the NaCl level of culture solution increased to 1.5%, the growth of *L. racemosa* seedlings decreased (Table 2), but leaves appeared green and healthy. The nomogram of seedlings in 1.5% NaCl was very similar to the 0.75% treatment, but displayed a longer C-shift magnitude for Cl and Na ions (Fig. 3A). Absorbing Cl and Na ions consumed a large quantity of energy, for example Cl ions accumulation by the root cells of halophytes accounts for over one fifth of their total ATP production (Yeo, 1983). The reduction in growth of mangrove seedlings with an increasing salinity of 25-50% seawater could be largely attributed to an increasing accumulation of the Na and Cl ions that required for osmotic adjustment (Clogh, 1984; Hwang and Chen, 1995). Therefore, this

study infers that this is the major limiting factor of *L. racemosa* growth in 1.5% NaCl salinity. However, vector analysis also displayed P in the leaves as deficient elements. Although the N, Mg, K and Ca displayed dilution effects that would not limit the growth of seedlings (Fig. 3B), but severe dilution also resulted in a secondary nutrient deficiency (Timmer, 1991). Larger magnitudes of these nutrients in comparison with 0.75% treatment attribute to a higher leaf succulence in this salinity (Table 2).

Most mangrove seedlings grew in 100% seawater salinity (approximately 3.0-3.5% NaCl), and the high concentrations of ions accumulated in the plant tissues caused a distinct reduction of growth (Mizrachi *et al*., 1980; Burchett *et al*., 1984; Hwang and Chen, 1995; Koch and Snedaker, 1997). The growth of *L. racemosa* seedlings treated with 3.0% NaCl was suppressed (Table 2) and the relative leaf dry weight in comparison with the control set was sharply reduced (Table 3). Under vector analysis, the contents and concentrations of Na and Cl increased but the growth rate decreased, implies that an excessive uptake of these ions caused toxicity in seedlings (Fig. 4A). Suárez *et al*. (1998) suggested that the cell volume of *Avicennia germinans* under high salinity might be changed by the solute concentration when the solute amount per cell remains unchanged. The osmotic potential could be lowered when the plant in the absorption of larger amounts of water. These physiological limits to the intracellular concentrations of ions reached more rapidly in leaves with smaller cells than in leaves with larger cells (Suárez e*t al*., 1998). *L. racemosa* seedlings grown in high salinity may have the mechanism that shed older leaves with considerable amounts of salt to rid of excess salt.

By using a vector analysis of *L. racemosa* seedlings grown in 3.0% treatments, the P nutrient in the foliage displayed an excess accumulation as toxic effect (Fig. 4B). This also is attributed to a decrease in leaf area and cell volume (Table 2). Although the magnitude remained comparable to the control set, this indicates that the toxic effect for seedlings were not obvious. Generally, Na attributes a decrease in the nitrogen uptake to the competitive inhibition of ammonium ions in mangrove habitat (Naidoo, 1987; Ball, 1996). However, Cl ion has an antagonistic effect on NO_3 accumulation (Hu and Schmidhalter, 1997; Grattan and Grieve, 1999). In addition, NaCl reduces plasmalemma-adenosine triphosphatase activity, which results in a suppression of NO_3 uptake (Hawkins and Lips, 1997). A decrease in K uptake in salt-treated plants is attributed to an exchange between Na and K at the xylem/symplast boundary of plant roots (Lacan and Durand, 1996), or as Na competes with K entry in membrane under saline conditions (Marschner, 1997). However, when plants are challenged with salinity stress, Ca is displaced by Na in the plasma membrane (Rausch *et al*., 1996). Increasing salinity reduces the concentrations of Mg in leaves (Ruiz *et al*., 1997; Wang *et al*., 1997), due primarily to ion competition by Na. Ca is also strongly competitive with Mg (Marschner, 1997). The above interactions between salt ions and mineral nutrients support this study's findings that the antagonism effects of N, Mg, K and Ca nutrients can be attributed to the excess accumulation Na or Cl. Clough (1984), Naidoo (1987) also conclude that the concentrations of these essential nutrients in mangrove seedlings decrease with a high salinity, resulting in a

reduction in growth. Therefore, in addition to the toxic effects incurred through high concentrations of Cl and Na, and an imbalance of essential nutrients contributed to a reduction in the growth of *L. racemosa* seedlings.

L. racemosa seedlings completely deadlocked when the salinity was increased to 4.5% and 6.0% hapersaline treatments (Table 2) and died by the end of the experiment. The vector diagnosis of nutrient in the foliage was similar to that of 3.0% NaCl treatments, and the longer magnitudes indicated the severe toxic and antagonistic effects of these nutrients (Figures 5A, B). Generally, the accumulation of excess ions in the cytoplasmic compartments would lead to metabolic dysfunction and cell death of mangrove species (Ball, 1996). Studies on mangrove seedlings grown at hypersaline conditions greater than 4.5% also had similar results (Tomlinson, 1994; Hwang and Chen, 1995; Koch and Snedaker, 1997).

V. Conclusion

Experimental results indicated that the 0.75% NaCl concentration was the optimum salinity for *L. racemosa* seedlings, and hypersaline conditions greater than 3.0% displayed toxic effects. This study employed a vector analysis and the diagnoses of each nutrient in different salinity treatments matched the visual symptoms, growth and nutrient responses. Vector analysis improved the detection of nutrient interactions, ionic balances and dilution/ accumulation effects under salt stress as conventional plant diagnosis might be confounded.

VI. Literature cited

Alarcón, J. J., Sanchez-Blanco, M. J., Bolarin, M. C. and Torrecillas, A. (1993) Water relations and osmotic adjustment in *Lycopersicon esculentum* and *L. pennellii* during short-term salt exposure and recovery. Physiol. Plant. 89:441-447.

Ball, M. C. (1988) Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina.* I. Water use in relation to growth, carbon partitioning, and salt balance. Aust. J. Plant Physiol. 15:447-464.

Ball, M. C. (1996) Comparative ecophysiology of mangrove forest and tropical lowland moist rainforest. P. 461-496. In Mulkey, S. S., Chazdon, R. L. and Smith, A. P. eds. Tropical Forest Plant Ecophysiology. Chapman and Hall, New York. pp.675.

Ball, M. C. and Anderson, J. M. (1986) Sensitivity of photosystem II to NaCl in relation to salinity tolerance. Comparative studies with thylakoids of the salt-tolerant mangrove, *Avicennia marina*, and the salt-sensitive pea, *Pisum sativum*. Aust. J. Plant Physiol. 13:689-698.

Brække, F. H. (1996) Needle analyses and graphic vector analyses of Norway spruce and Scots pine stands. Trees 11:23-33.

Burchett, M. D., Field, C. D. and Pulkownik, A. (1984) Salinity, growth and root respiration in the grey mangrove, *Avicennia marina*. Physiol. Plant. 60:113-118.

Clough, B. F. (1984) Growth and salt balance of the mangroves *Avicennia marina* (Forsk.) Vierh. and *Rhizophora stylosa* Griff. in relation to salinity. Aust. J. Plant Physiol. 11:419-430.

Downton, W. J. S. (1982) Growth and osmotic relations of the mangrove *Avicennia marina*,

as influenced by salinity. Aust. J. Plant Physiol. 9:519-528.

Duke, N. C. (1992) Mangrove floristics and biogeography. P. 63-100. In Robertson A. L. and Alongi, D. M. eds. Coastal and Estuarine Studies: Tropical Mangrove Ecosystems. American Geophysical Union, Washington, DC. pp.330.

Fan, K. C., Sheu, B. H. and Chang, C. T. (1999) Effects of soil salinity on water status, chlorophyll fluorescence and cell viability of *Limnitzera racemosa* seedlings. Q. J. Chin. For. 32:469-480. [in Chinese with English summary].

Fan, K. C. (2000) Effects of soil salinity on the growth and physiological responses of the mangrove *Lumnitzera racemosa* seedlings. [Ph.D thesis] Taichung, Taiwan: Department of Forestry, National Chung-Hsing Univ. pp.128 . [in Chinese with English summary].

Ghosh, G. and Drew, M. C. (1991) Comparison of analytical methods for extraction of chloride from plant tissue using ^{36}Cl as tracer. Plant and Soil. 136:265-268.

Grattan, S. R. and Grieve, C. M. (1999) Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic. 78:127-157.

Gucci, R., Lombardini, L. and Tattini, M. (1997) Analysis of leaf water relations in leaves of two olive (*Olea europaea*) cultivars differing in tolerance to salinity. Tree Physiol. 17:13-21.

Haase, D. L. and Rose, R. (1995) Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. For. Sci. 41:54-66.

Hawkins, H. J. and Lips, S. H. (1997) Cell suspension cultures of *Solanum tuberosum* L. as a model system for N and salinity response effect of salinity on NO_3^- uptake and PM-ATPase activity. J. Plant Physiol. 150:103-109.

Hoagland, D. R. and Arnon, D. I. (1950) The Water – Culture Method for Growing Plants without Soil. Calif. Agric. Expt. Sta. Circ. 347 pp.

Hu, Y. and Schmidhalter, U. (1997) Interactive effects of salinity and macronutrient level on wheat. II.Composition. J. Plant Nutri. 20:1169-1182.

Huang, S., Shih, J. T. and Hsueh, M. L. (1998) Mangroves of Taiwan. Taiwan Endemic Species Research Institute, pp.176.

Hwang, Y. H. and Chen, S. C. (1995) Salt tolerance in seedlings of the mangrove *Kandelia candel* (L.) Druce, Rhizophoraceae. Bot. Bull. Acad. Sin. 36:25-31.

Imo, M. and Timmer, V. R. (1997) Vector diagnosis of nutrient dynamics in mesquite seedlings. For. Sci. 43:268-273.

Imo, M. and Timmer, V. R. (1999) Vector competition analysis of black spruce seedling responses to nutrient loading and vegetation control. Can. J. For. Res. 29:474-486.

Johnson, C. M., Stout, P. R., Broyer, T. C. and Carlton, A. B. (1957) Comparative chlorine requirements of different plant species. Plant and Soil 8:337-353.

Kalra, Y. P. and Maynard, D. G. (1991) Methods Manual for Forest Soil and Plant Analysis. Northwest Region Information Report NOR-X-319. Forestry Canada, Northwest Region, Northern Forestry Centre. pp.116.

Kiefer, J. W. and Fenn, M. E. (1997) Using vector analysis to assess nitrogen status of ponderosa and Jeffrey pine along deposition

gradients in forests of southern California. For. Ecol. Manage. 94:47-59.

Koch, M. S. (1997) *Rhizophora mangle* L. Seedling development into the sapling stage across resource and stress gradients in subtropical Florida. Biotropica 29:427-439.

Koch, M. and Snedaker, S. C. (1997) Factors influencing *Rhizophora mangle* L. seeding development in Everglades carbonate soils. Aquat. Bot. 59:87-98.

Kozlowshi, T. T. (1997) Response of woody plants to flooding and salinity. Tree Physiology Monograph No 1. pp.17.

Lacan, D. and Durand, M. (1996) Na^+-K^+ exchange at the xylem/symplast boundary. Plant Physiol. 110:705-711.

Lin, G. and Sternberg. L. da S. L. (1992) Comparative study of water uptake and photosynthetic gas exchange between scrub and fringe red mangrove (*Rhizophora mangle* L.). Oecologia 90:399-403.

Macdonald, S. E., Margaret, G. S. and Rothwell, R. L. (1998) Impacts of mechanical site preparation on foliar nutrients of planted white spruce seedlings on mixed-wood boreal forest sites in Alberta. For. Ecol. Manage. 110:35-48.

Mallery, C. H. and Teas, H. J. (1984) The mineral ion relations of mangrove. I. Root cell compartments in a salt excluder and a salt secreter species at low salinities. Plant Cell Physiol. 25:1123-1131.

Marschner, H. (1997) Mineral Nutrient of Higher Plants, 2nd edn. Academic Press, London. pp.889.

Mizrachi, D., Pannier, R. and Pannier, F. (1980) Assessment of salt resistance mechanisms as determinant physio-ecological parameters of zonal distribution of mangrove species. I. Effect of salinity stress on nitrogen metabolism balance and protein synthesis in the mangrove species *Rhizophora mangle* and *Avicennia nitida*. Bot. Mar. 23:289-296.

Naidoo, G. (1985) Effects of waterlogging and salinity on plant-water relations and on the accumulation of solutes in three mangrove species. Aquat. Bot. 22:133-143.

Naidoo, G. (1987) Effects of salinity and nitrogen on growth and water relations in the mangrove, *Avicennia marina* (Forsk.) Vierh. New Phytol. 107:317-325.

Popp, M., Polania, J. and Weiper, M. (1993) Physiological adaptations to different salinity levels in mangrove. P. 217-224. In Lieth, H. and Al Masoom, A. A. eds. Towards the Rational Use of High Salinity Tolerant Plants, Vol.1. Kluwer cademic Publishers, Dordrecht, Netherlands. 521 pp.

Proe, M. F., Craig, J., Dutch, J. and Griffiths, J. (1999) Use of vector analysis to determine the effects of harvest residues on early growth of second-rotation Sitka spruce. For. Ecol. Manage. 122:87-105.

Rausch, T., Kirsch, M., Löw, R., Lehr, A., Viereck, R. and Zhigang, A. (1996) Salt stress responses of higher plants: The role of proton pumps and Na^+/H^+-ntiporters. J. Plant Physiol. 148:425-433.

Ruiz, D., Martinez, V. and Cerda, A. (1997) Citrus response to salinity: growth and nutrient uptake. Tree Physiol. 17:141-150.

Smith, S. M. and Snedaker, S. C. (1995) Salinity responses in two populations of viviparous *Rhizophora mangle* L. seedlings. Bioropica 27:435-440.

Suárez, N., Sobrado, M. A. and Medina, E.

(1998) Salinity effects on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) L. seedlings. Oecologia 114:299-304.

Timmer, V. R. and Stone, E. L. (1978) Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Soil Sci. Soc. Am. J. 42:125-130.

Timmer, V. R. and Armstrong, G. (1987) Diagnosing nutritional status of containerized tree seedlings: Comparative plant analysis. Soil Sci. Soc. Am. J. 51:1082-1086.

Timmer, V. R. (1991) Interpretation of seedling analysis and visual symptoms. P. 113-134. In van den Driessche, R. eds. Mineral Nutrition of Conifer Seedlings. CRC Press, Boca Raton, Florida. pp.274.

Tomlinson, P. B. (1994) The Botany of Mangroves, 2nd ed. Cambridge University Press, New York. pp.419.

Wang, L. W., Showalter, A. M. and Ungar, I. A. (1997) Effects of salinity on growth, ion content, and cell wall chemistry in *Atriplex prostrata* (Chenopodiaceae). Am. J. Bot. 84:1247-1255.

Weetman, G. F., McDonald, M. A., Prescott, C. E. and Kimmins, J. P. (1993) Responses of hemlock, Pacific silver fir, and western red cedar plantations on northern Vancouver Island to applications of sewage sludge and inorganic fertilizer. Can. J. For. Res. 23:1815-1820.

Werner, A. and Stelzer, R. (1990) Physiological responses of the mangrove *Rhizophora mangle* grown in the absence and presence of NaCl. Plant Cell Environ. 13:243-255.

Yeo, A. R. (1983) Salinity resistance: Physiologies and prices. Physiol. Plant. 58: 214-222.