Effects of canopy cover and seasonal reduction in rainfall on leaf phenology and leaf traits of the fern *Oleandra pistillaris* in a tropical montane forest, Indonesia

Running head: Leaf phenology and leaf traits of *Oleandra pistillaris*

Key words: fern, leaf life span, leaf mass per area, open conditions, stem growth, stable carbon isotope ratio, stomatal density, understorey conditions, water use efficiency

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Abstract: Leaf phenology and leaf traits of the fern *Oleandra pistillaris* were examined in relation to canopy cover (open and understorey) and seasonal reduction in rainfall in a wet tropical montane forest, Indonesia. Although the annual rainfall is high, rainfall is relatively less in June and July. Stomatal density and diameter were greater in the open than in the understorey (229 versus 167 mm^{-2} for stomatal density and 33 versus 29 μm for stomatal diameter). The stable carbon isotope ratio ($\delta^{13}C$) of leaves, positively correlated with water use efficiency, was higher in the open than in the understorey (mean $\delta^{13}C$ –30 versus –33‰). Therefore, it is considered that leaves have high gas-exchange capacities per leaf area in the open where water availability would be limited, compared with the understorey. In contrast, leaf mass per area (LMA) was lower and leaf life span was longer in the understorey than in the open (25 versus 34 g m^{-2} for LMA and 2.1 and 1.6 y for leaf life span). These thin leaves with a long life span in the understorey would contribute to efficient light capture and photosynthetic production per leaf mass. The number of leaves per stem decreased during the period with less rainfall in both the open and understorey conditions, which should reduce the water loss from plants, but increased again after the period with less rainfall. Stem growth rate was higher in the open than in the understorey, and the seasonal reduction in rainfall hardly affected stem growth rate   open   understorey conditions. This study concludes that *O. pistillaris* responds to canopy cover and seasonal reduction in rainfall by adjusting leaf traits and leaf phenology, respectively.

INTRODUCTION

Light is one of the most important resources for growth and survival of understorey plants. Light conditions fluctuate in time and space, and therefore, plants must adapt to various light conditions. Leaves often change morphologically in response to light conditions. Generally, leaf mass per area (LMA) increases with light intensity. The construction of leaves in this way is attributable to a thick palisade layer, which increases the concentration of nitrogen-rich photosynthetic enzymes, such as RuBP carboxylase, per leaf area, thus increasing the assimilative capacity of leaves per leaf area (Ellsworth & Reich 1993, Niinemets *et al*. 1999, Takahashi *et al*. 2005). Stomatal density often increases with light intensity (Abrams & Kubiske 1990), resulting in higher gas-exchange rates.

Drought stress often decreases size growth rates of plants (Newbery & Lingenfelder 2004), and increases water use efficiency (the ratio of mass CO_2 fixed to mass H_2O transpired) that is positively correlated with the stable carbon isotope ratio ($\delta^{13}C$) of leaves and stems (Ferrio *et al*. 2003, Lauteri *et al*. 1997, Van de Water *et al*. 2002, Warren *et al*. 2001). Plants respond phenologically to seasonal reduction in rainfall. For example, in forests where dry periods are common regular both leaf-flushing and leaf-shedding tend to be seasonal, with leaf-shedding confined mainly to the relatively dry period (Nobel 1978, Richards 1996, Sharpe 1997), to prevent water loss. Therefore, plants respond to light conditions and seasonal reduction in rainfall both morphologically and phenologically.

Oleandra pistillaris (Sw.) C. Chr. (Oleandraceae) is a terrestrial and facultative hemi-epiphytic fern species in tropical montane forests in South-East Asia. Terrestrial *O.*

pistillaris grows mainly at open sites. The climate of the Bogor region in western Java

in Indonesia is characterized by wet conditions with no dry month (monthly rainfall <

100 mm). Nevertheless, a seasonal reduction in rainfall regularly occurs in this region

(Nakamura *et al*. 1994). Therefore, seasonal reduction in rainfall might affect the

growth and leaf phenology of *O. pistillaris* growing in this region.

The purpose of this study was to examine how *O. pistillaris* adapts to canopy

conditions (open and understorey) and to seasonal reduction in rainfall in a tropical

montane forest, Java, Indonesia. For this purpose, leaf traits, such as number of leaves

per stem, leaf mass per area, stomatal density and size, and $\delta^{13}C$, were compared

between open and understorey conditions, and leaf phenology and stem growth were

examined in relation to seasonal reduction in rainfall.

STUDY SITE

This study was carried out in a tropical forest in Gunung Halimun National Park (6˚ 44'

S, 106˚ 32' E, 1200 m asl), West Java, Indonesia, from March 2003 to March 2004.

Dominant tree species were *Altingia excelsa* Noronha, *Castanopsis acuminatissima* A.

DC. ex Hance, *Schima wallichii* Choisy and *Quercus lineata* Blume (Suzuki *et al*. 1997).

Several ginger species (*Alpinia* spp.) dominated the understorey vegetation (Nishimura

& Suzuki 2000, Takahashi 2004).

The monthly mean temperatures, recorded at the office of a tea plantation near

the study site (ca. 2 km from the study site in horizontal distance), were between 25.8˚C

and 29.5˚C during 1998 and 1999 (Figure 1). The annual mean rainfall was 3869 mm

from 1998 to 2002. Monthly rainfall exceeds 150 mm, even in the driest month of July

(Figure 1). Monthly potential evapotranspiration, calculated by Thornthwaite model (Thornthwaite 1948), ranges between 130 and 160 mm. Although rainfall exceeded potential evapotranspiration in each month, rainfall of July was close to the potential evapotranspiration of this month (Figure 1).

MATERIALS AND METHODS

Oleandra has a creeping rhizome with erect, branched stems. The leaves are simple, entire and clustered in pseudowhorles with long internodes (Nayar & Bajpai 1976, Tryon 1997, 2000). Two or three leaf clusters are found along the stem (Figure 2). It regenerates vegetatively by rhizomes.

An open site (ca. 1500 m^2) on a ridge was chosen for measurements. This open site was not a treefall gap that was recently formed because there were no fallen logs. Another site was also selected in the understorey conditions. The distance from the open site was about 60 m. Eleven quadrats of 1×0.5 m and 10 quadrats of 2×2 m were set up at the end of March 2003 in open and understorey conditions, respectively. Stem density was assessed by measuring the number and length of stems in each quadrat. The presence of sori was also checked for each stem. No climbing *O. pistillaris* was found in any quadrat.

In October 2003, 30 and 25 stems were randomly chosen in the understorey and open, respectively. One leaf was sampled from each stem. Stomatal density, stomatal diameter, LMA and $\delta^{13}C$ were determined for each leaf. The stomatal frequency was estimated from nail varnish replicas of the abaxial surface at three points on each leaf. The number of stomata was counted at each point, using a microscope

with a lattice micrometer. The diameter of three stomata was measured on each leaf. The leaf area was measured, using a scanner and a Macintosh computer with the public domain NIH image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The leaves were oven-dried at 80˚C for at least 48 h and weighed before LMA was calculated for each leaf. Then the leaves were ground into a powder, and the $\delta^{13}C$ was measured, using a mass spectrometer (DELTA plus, ThermoQuest Ltd, Yokohama, Japan) with an elemental analyzer (Flash EA1112, ThermoQuest Ltd, Thermo Electron Ltd, Yokohama, Japan). The $\delta^{13}C$ of leaves is often positively correlated with its LMA (Hultine & Marshall 2000, Niinemets *et al*. 1999, Vitousek *et al*. 1990), because a high LMA (or thick leaves) decreases the mesophyll conductance of CO_2 diffusion from the stomatal cavities to the carboxylation sites. Therefore, $\delta^{13}C$ was compared between open and understorey in relation to LMA.

To examine stem growth in relation to seasonal reduction in rainfall, 32 and 30 stems were randomly chosen from the open and understorey, respectively, at the end of March 2003. These stems were tagged for repeated censuses. The three leaf clusters were numbered from the top downward (Figure 2). The number of leaves in each leaf cluster was counted and the stem length was measured. This was repeated in early October 2003 and the end of March 2004; March and October were before and after the driest month of July, respectively (Figure 1). New immature leaf clusters occurred at the top of many stems in October 2003. However, these leaves were not counted because they were immature and small. These leaves had fully developed by March 2004 and were named cluster 0.

The leaf life span of *O. pistillaris* was calculated by the following equation

(King 1994).

$$\text{Leaf life span (y)} = \frac{2 \times (\text{mean number of live leaves})}{\text{leaf production rate} + \text{leaf loss rate}}$$

The number of leaves in March 2003 and March 2004 was used for the calculation. Of the 62 stems in the open and understorey, the apical meristems of eight stems died during the census period (i.e. these stems could not produce new leaves), these stems were excluded from the calculation of the leaf life span.

RESULTS

Mean stem density was 3.7 times higher in the open (27.6 stems m^{-2}) than in the understorey (7.5 stems m^{-2}) (ANOVA, $F_{1, 19} = 83.5$, $P < 0.001$). The mean stem length ($\pm$ SD) was greater in the open (85 ± 29 cm) than in the understorey (62 ± 28 cm) (ANOVA, $F_{1, 448} = 77.1$, $P < 0.001$, Figure 3). The percentage of stems with sori was greater in the open (41%) than in the understorey (9%) (Figure 3). The number of leaves per stem was also greater in the open than in the understorey, because this number was positively correlated with stem length (data not shown).

The LMA (ANOVA, $F_{1,53} = 21.7$, $P < 0.001$), stomatal density (ANOVA, $F_{1,53} = 62.9$, $P < 0.001$) and stomatal diameter (ANOVA, $F_{1,53} = 9.1$, $P < 0.01$) of the *O. pistillaris* leaves were greater in the open than in the understorey (Table 1). The $\delta^{13}C$ of leaves positively correlated with the LMA in the open ($r = 0.59$, $P = 0.002$), but this correlation was not significant in the understorey ($r = 0.26$, $P = 0.16$, Figure 4). Although the $\delta^{13}C$ dependency on LMA differed between the open and understorey, the mean value of $\delta^{13}C$ was higher in the open (−29.9‰) than in the understorey (−32.6‰)

(Mann-Whitney U-test, U = 692, P < 0.001, Figure 4).

The number of leaves per stem fluctuated from March 2003 to March 2004 in both the understorey and open (Figure 5). At the open site, mean total number of leaves per stem decreased from 17.5 in March 2003 (before the seasonal reduction in rainfall), to 9.9 in October 2003 (after the seasonal reduction in rainfall). However, this value increased to 14.4 by March 2004 (Figure 5). The same tendency was also noted in the understorey: the total number of leaves per stem was, on average, 10.6 in March 2003, 5.7 in October 2003, and 9.8 in March 2004 (Figure 5). The reduction in number of leaves from April to October 2003 was due to leaf shedding (Figure 5). The increase in leaf number from October 2003 to March 2004 was due to the emergence of new leaves at cluster 0. At the open site, the number of leaves at cluster 1 decreased, from 14.2 in March 2003, to 9.7 in October 2003, and to 4.7 in March 2004 (Figure 5). During this time, however, there was no significant decrease at cluster 1 in the understorey leaf number (Figure 5). Estimated leaf life span was longer in the understorey (2.1 y) than in the open (1.6 y) (ANOVA, $F_{1,53} = 13.7$, P < 0.001).

tem growth rate was higher in the open than in the understorey during April to September (U = 128, P < 0.001) and during October to March (U = 91, P < 0.001, Figure 6). However, the stem growth rate did not significantly differ between the two periods in the open and understorey.

DISCUSSION

In both the open and understorey, leaves of *Oleandra pistillaris* were shed during the period of April to September that included the seasonal reduction of rainfall in June and

July, and new leaves emerged after the seasonal reduction in rainfall. Such leaf phenology is considered to be adaptive to seasonal reduction in rainfall, because the reduction of transpiring leaf area should decrease the water loss from plants. Many studies also found that trees often shed leaves during the dry season and start new leaf growth after the start of the rainy season (de Bie *et al*. 1998, Harrison *et al*. 2000, Prins 1988, Richards 1996, Tanner 1983). Higher $\delta^{13}C$ of *O. pistillaris* leaves in the open than in the understorey indicates water availability would be more limited in the open than in the understorey. This may have resulted from l soil water availability due to high evapotranspiration caus stomatal closure of *O. pistillaris* in the open (cf. Bréda *et al*. 1995, Cienciala *et al*. 1998). Reich & Borchert (1984) observed that girth increment is much greater in the rainy season than in the dry season for several tree species in a tropical lowland forest in Costa Rica, where rainfall was low or negligible during the dry season. However, the stem growth rate of *O. pistillaris* differed little between the two periods, even in the open. The monthly rainfall was greater than 100 mm at our study site, even during the driest month of July. Therefore, the seasonal reduction in rainfall actually increased the water use efficiency in the open, but may not be severe enough to reduce the stem growth rate of *O. pistillaris* in both the open and understorey.

The LMA, stomatal density and diameter of *O. pistillaris* leaves were greater in the open than in the understorey. Generally, LMA increases with light intensity, as occurs in open conditions. Thickness of palisade layer increases in conditions, which improves the assimilative capacity per unit leaf area (Ellsworth & Reich 1993, Gould 1993, Niinemets *et al*. 1999, Takahashi *et al*. 2005, 2006). The greater stomatal density and diameter of *O. pistillaris* should lead to greater CO_2 diffusion per unit leaf area in the open. Other researchers have also observed that stomatal density is higher in the

open than in the understorey, and that stomatal size is as large or larger in the open than in the understorey (Abrams & Kubiske 1990, Abrams & Mostoller 1995, Ashton & Berlyn 1994). Therefore, *O. pistillaris* had leaves with higher gas-exchange capacity in the open than in the understorey.

Leaf life span was longer and LMA lower in the understorey than in the open. For an individual leaf, acclimation to shade can be explained by leaf life span and LMA: plants grown under suppressed understorey conditions generally have thinner leaves (Ellsworth & Reich 1993, Takahashi *et al*. 2001) and leaves with a longer life span (Kikuzawa 1988). The low LMA and long life span of leaves increase lifetime light capture per unit leaf mass, which increase photosynthetic production per unit leaf mass. Therefore, leaves of *O. pistillaris* plastically responded to open and understorey conditions to increase light capture efficiency and photosynthetic production.

The results of this study indicate that *O. pistillaris* responds to seasonal reduction in rainfall by adjusting leaf phenology and adapts to both open and understorey conditions by adjusting leaf traits, such as LMA, stomatal density and diameter and leaf life span. Therefore, *O. pistillaris* responds to seasonal reduction in rainfall and differences in light intensity phenologically and morphologically.

ACKNOWLEDGEMENTS

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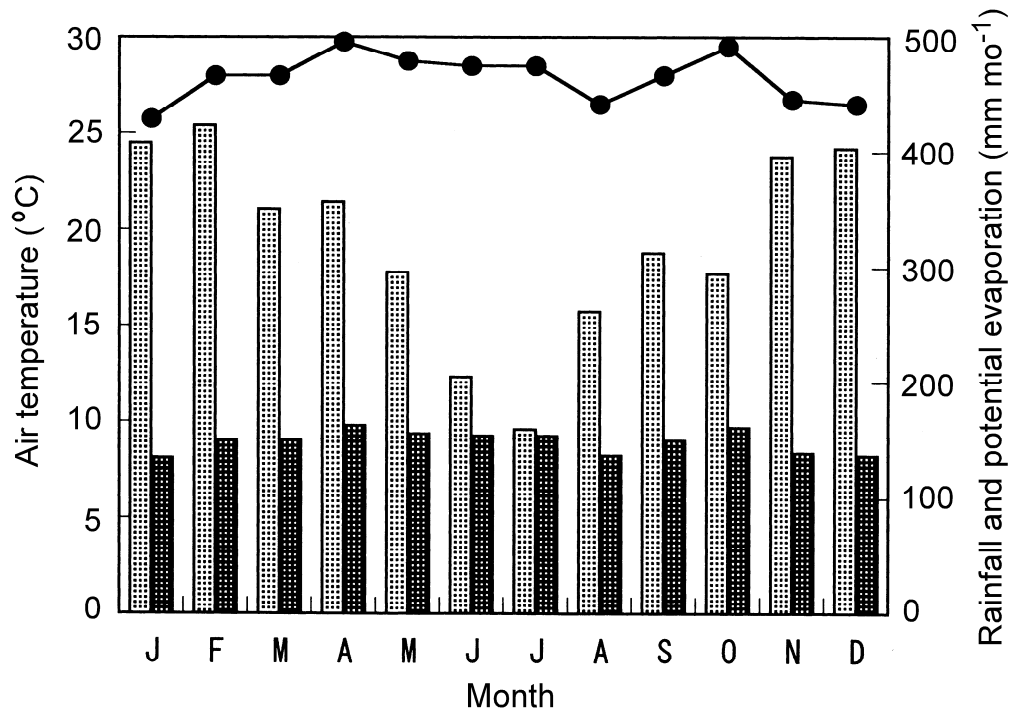
Figure 1. Monthly mean air temperatures (circles), monthly sum of rainfall (light shaded bars) and monthly sum of potential evapotranspiration (dark shaded bars), recorded near the study site (ca. 2 km from the study site) in West Java, Indonesia.
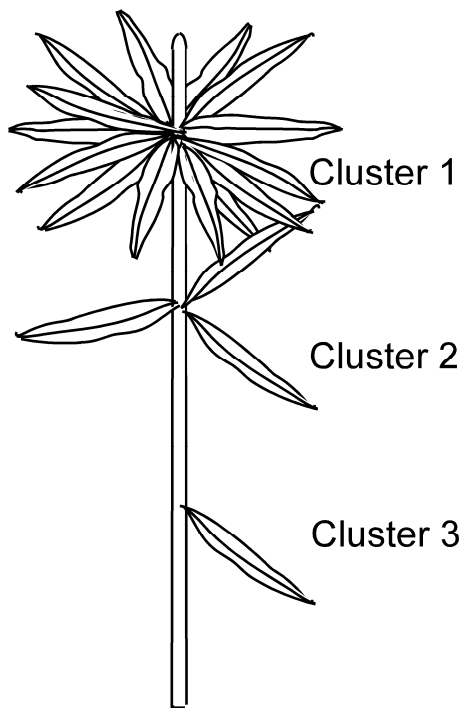
Figure 2. Open-grown *Oleandra pistillaris* in March 2003. Leaves are clustered in pseudowholes with long internodes. In March 2003, each leaf cluster was named cluster 1, 2 or 3 from the top. A new leaf cluster at the top of stems in March 2004 was named as cluster 0.
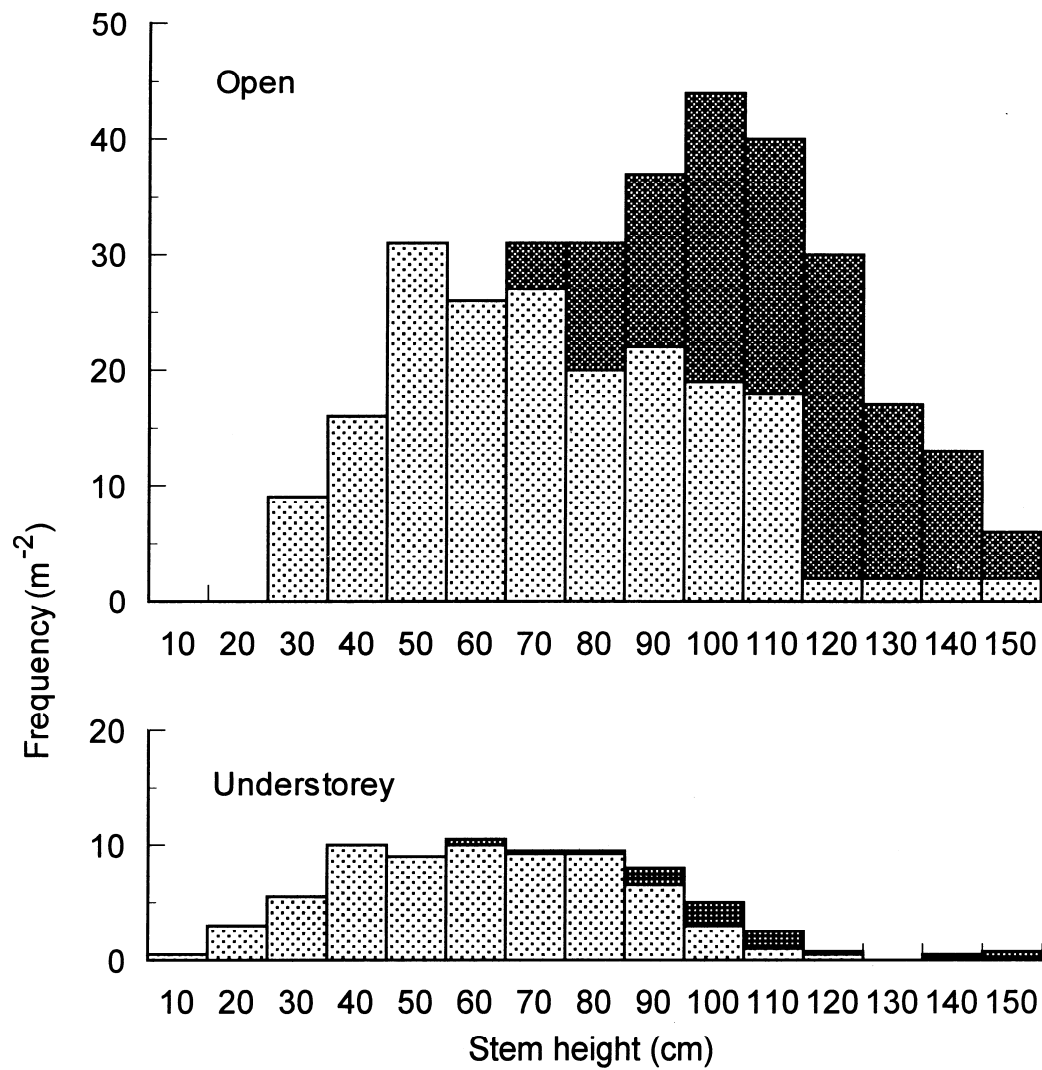
Figure 3. Frequency distribution of the stem length of *Oleandra pistillaris* in the open and understorey, in March 2003. Dark and light shaded bars indicate stems with and without sori, respectively.
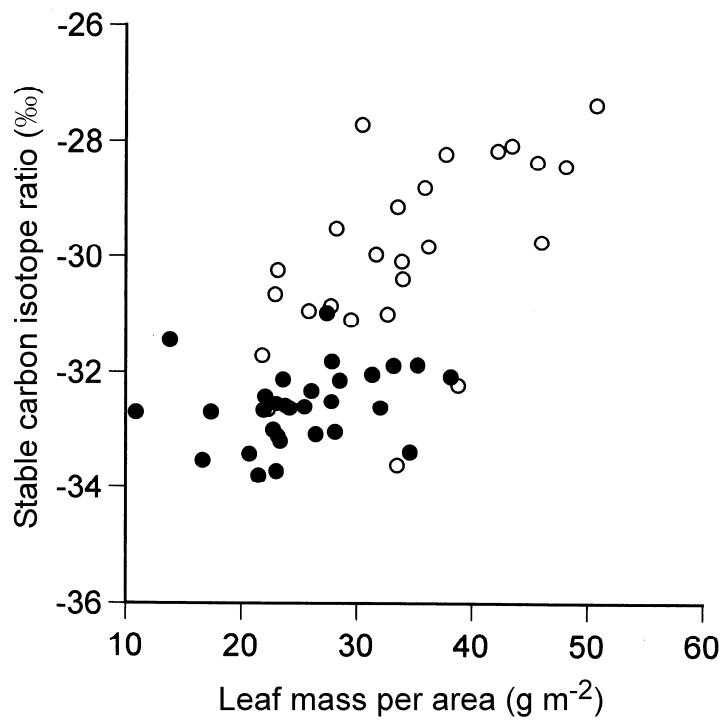
Figure 4. Relationship between leaf mass per area and the stable carbon isotope ratio of leaves of *Oleandra pistillaris*. Open and solid circles are open and understorey, respectively.
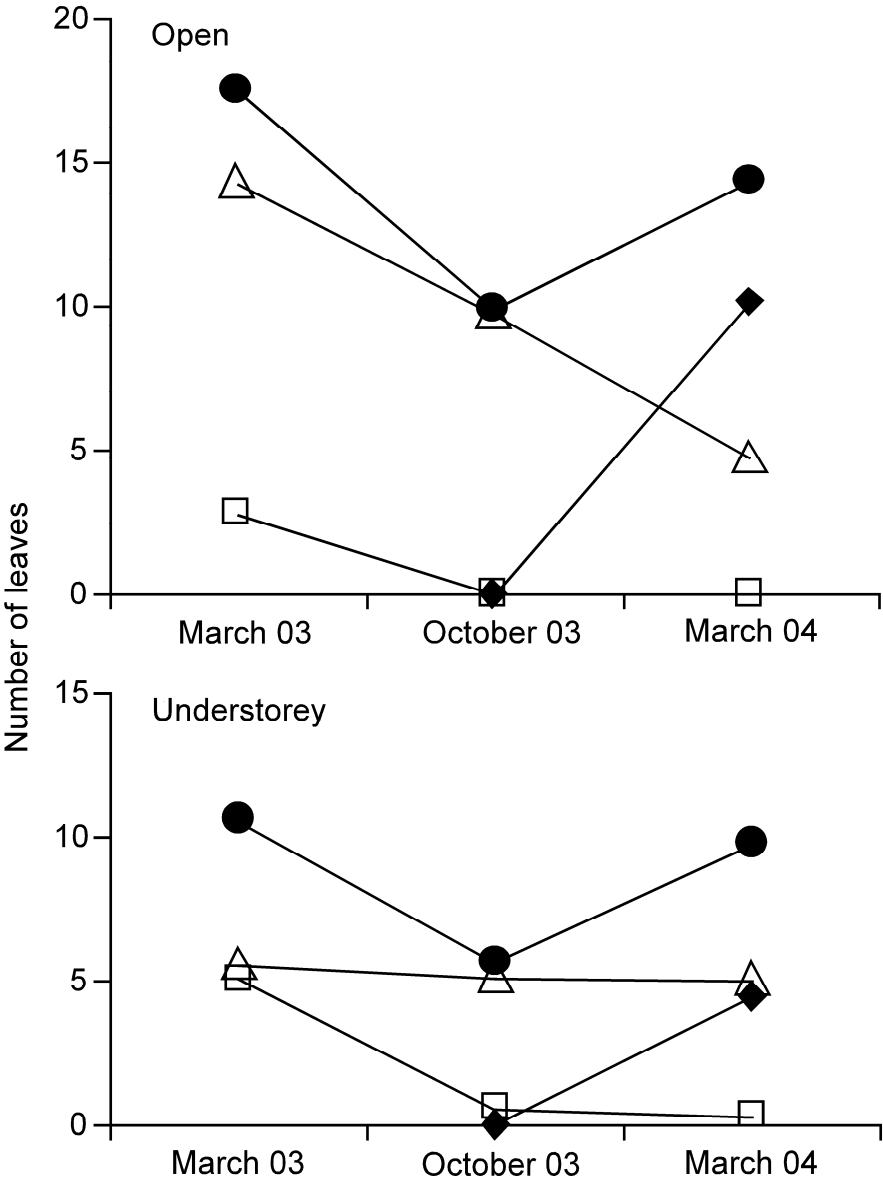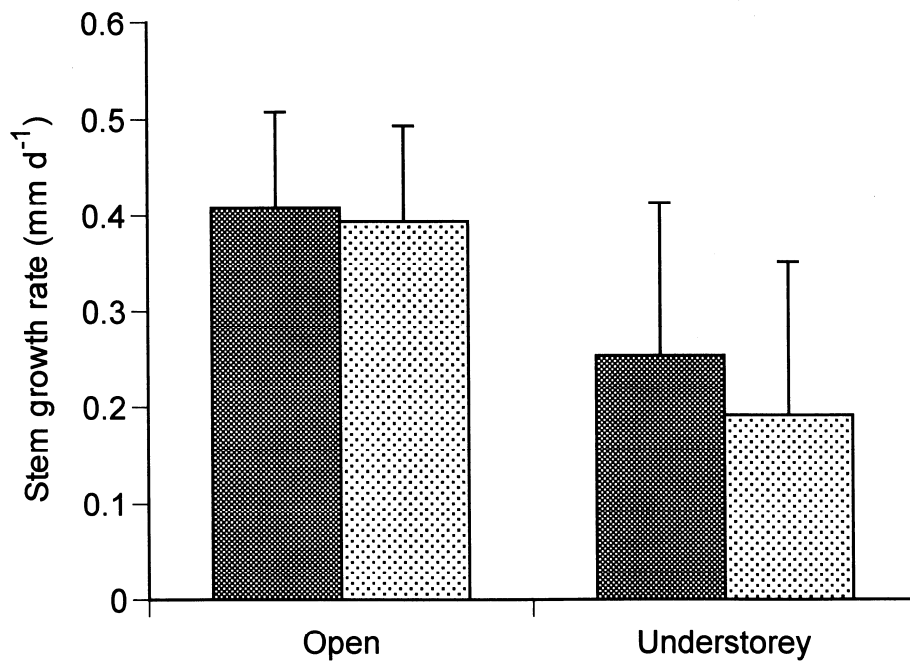
Figure 5.

Figure 6. Stem growth rates of *Oleandra pistillaris* in the open and understorey. Dark and light shaded bars are the stem growth rates during April to September and during October to March, respectively. The stem growth rate is shown with its positive standard deviation.

Table 1. Leaf mass per area, stomatal density and diameter of *Oleandra pistillaris* sampled in the understorey and open conditions. Mean± SD.

Leaf traits	Open (n = 25)	Understorey (n = 30)	F-value (df = 1, 53)
Leaf mass per area (g m^{-2})	34.2 ± 8.3	25.1 ± 6.2	21.7[***]
Stomatal density (no. mm^{-2})	229 ± 42.7	167 ± 27.8	62.9[***]
Stomatal diameter (μm)	32.7 ± 6.1	28.7 ± 3.8	9.1[**]

**, $P < 0.01$; ***, $P < 0.001$ by ANOVA.