THE INFLUENCE OF THE MONOCARPIC HERB, *ISOGLOSSA WOODII,* ON SUBTROPICAL FOREST TREE DYNAMICS AND DIVERSITY

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ABSTRACT

Dominant understorey species, such as herbs, ferns, palms and shrubs may influence forest tree species diversity and dynamics. Their influence may be through shading the forest floor, thereby affecting regeneration of shade-intolerant species and reducing species diversity, or it may be through competition with seedlings for space and belowground resources, thus modifying or changing the structure of the forest. These effects may be compounded if the life cycle of the understorey species consists of synchronized reproductive and mortality events. This study examines the influence of a dominant understorey species, *Isoglossa woodii* (Acanthaceae), on regeneration of trees in Indian Ocean subtropical coastal dune forest in southern Africa. The species is a semi-woody herb and has population-wide synchronous reproduction at 4-7 year cycles after which it dies and regenerates from seed. In this thesis I examine three aspects of the ecology of this suppressive herb: (i) the ecological and environmental correlates of the distribution of *I. woodii*; (ii) the evolutionary advantages of synchronous monocarpy; and (iii) the ecological effects of the extensive cover and putative recruitment window caused by *I. woodii* on forest tree seedling dynamics and diversity.

Isoglossa woodii covered 65–95 % of the understorey, while gaps in this understorey cover occupied the remaining 5–35 % of the area. The spatial distribution of *I. woodii* was strongly related to tree canopy structure, with the species excluded from sites with dense canopy cover. Woody seedling establishment was inhibited by low light availability (< 1 % of photosynthetically active radiation) beneath *I. woodii* so that the tree seedling community beneath *I. woodii* was a subset of the seedling community in gaps, clearly indicating the suppressive nature of the herb stratum.

To test whether tree recruitment limitation may operate indirectly through provision by *I. woodii* of habitat to fruit and seedling consumers, fruit removal and seedling survival were compared in uncaged and caged treatments set in *I. woodii* gaps and thickets. The feeding behaviour of frugivores was not influenced by the presence of *I. woodii*. Tree seedlings had reduced survival in *I. woodii* thickets, indicating that factors other than fruit and seedling predation limit the recruitment of tree seedlings beneath the herb layer in these forests. These factors may include post-emergence processes such as competition with the herb for light and nutrients.

The competitive interaction between *I. woodii* and tree seedlings was investigated using four common tree species (*Apodytes dimidiata*, *Diospyros natalensis*, *Euclea racemosa* and *Sideroxylon inerme*) that were grown in the greenhouse and in the field with young clones of *I. woodii* under contrasting light and nutrient conditions. In the greenhouse, total seedling biomass decreased with density of *I. woodii* and light levels but not with site fertility. Relative growth rate of seedlings increased with nutrients, was light neutral and decreased with *I. woodii* density. The magnitude of biomass suppression with competitor density and light levels was similar among tree species. Consequently there was no discernable hierarchy of competitive ranking of the tree species. In the field, aboveground biomass of tree seedlings increased with density of *I. woodii*. Thus, the herb reduced tree seedling performance.

The pollination biology of *I. woodii* was examined during a mass-flowering event in 2007. The main pollinator of *I. woodii* flowers was the African honey bee *Apis mellifera adansonii* and no seed was set in the absence of pollinators. *Isoglossa woodii* has a mixed mating strategy, with similar levels of seed set among selfed and outcrossed flowers. Natural fruit set of 60 ± 2 % was not increased by supplemental pollinations, indicating that pollinators were not saturated. Although flowers contained four ovules, most fruits contained only one seed, raising the possibility that preferential outcrossing occurs by selective abortion of seeds. Through outcrossing, *I. woodii* benefits from reproductive synchrony but the benefits of geitonogamous selfing are not clear. However, selfing may be related to reproductive assurance and provision of larger seed crops than can be consumed by seed predators (the predator satiation hypothesis).

The seed predator satiation hypothesis was tested as an evolutionary advantage of synchronous reproduction in *I. woodii*. Seed production was not influenced by insect herbivory on floral parts, but was greater on the foredune than on dune crests (14134 vs 6142 seed m^{-2}, respectively). Predation of *I. woodii* seed was not related to background quantities of seed in the soil but retention of 50-74 % of seed on the soil surface after the seed fall period provides support for the predator satiation hypothesis. *I. woodii* seedlings transplanted to paired *I. woodii* gaps and *I. woodii* thickets had greater survivorship in the thickets than in the gaps. Mortality of post-reproductive plants facilitated seedling establishment while deep shade in understorey gap sites precluded *I. woodii* regeneration.

Satiation of seed predators and facilitation of seedling establishment through death of parent plants ensures local dominance of the herb in successive generations.

The effect of *I. woodii* on tree seedling establishment was tested over a 7-yr reproductive cycle of the herb using 19 10 m × 10 m permanent experimental plots established in 2001. Each plot contained four 5 m × 5 m sub-plots of which two were fenced to exclude large herbivores and the herb stratum was removed from one of each pair of fenced and unfenced sub-plots. Once each year the species composition, height and stem diameter of each tree seedling were measured in five 1-m^2 quadrats located in each sub-plot. The density and species composition of tree seedlings were greater in the *I. woodii*-removed than beneath intact *I. woodii* areas while herbivore effects were greater in the *I. woodii*-removed areas compared to areas with intact *I. woodii*. Both the herb and herbivore effects suppressed seedlings but the magnitude of suppression from the herb was greater. Thus, *I. woodii* suppresses tree regeneration through its effects on reducing density and species composition of seedlings. However, its absence in understorey gaps enhances patchy tree seedling establishment, and its monocarpic strategy provides tree seedlings with competitive release.

Light response characteristics of seedlings of the main tree species from the plots were measured in the laboratory. Seedlings of these species had low light compensation points (range 2.14 ± 0.84 to 6.36 ± 0.69 μmol m^{-2} s^{-1}), high apparent quantum yields and low rates of dark respiration, which indicates that the species are shade-tolerant.

This study shows that *I. woodii* is an important ecological filter in dune forests that influences seedling establishment through its differential influence on seedling abundance and diversity. The selective filter has resulted in phenotypic clustering of relatively shade-tolerant species in the forest while shade-intolerant species have been reduced over time. Regeneration of the shade-tolerant species is enhanced in the gaps and during the post-reproductive period of the herb. Thus, the distribution and life-history of *I. woodii* has important implications for forest regeneration, and ultimately for forest structure and composition.

PREFACE

The work presented in this thesis was carried out in the Forest Biodiversity Research Unit of the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg from May 2004 to December 2009, under the supervision of Professors Michael J. Lawes and Colleen T. Downs and co-supervision of Dr Megan E. Griffiths.

This thesis represents original work by the author and has not been submitted in any form to another university. Where use has been made of the work of others, it is duly acknowledged in the text.

The thesis was written as papers submitted or to be submitted for publication, except Chapter 1 (Introduction) and Chapter 9 (Summary). Chapters written as papers follow the format of the journal to which they were/will be submitted. The main difference is that tables and figures are in the text and not at the end of the each chapter.

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October 2009

We certify that the above statement is correct

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DECLARATION 1 - PLAGIARISM

I, Zivanai Tsvuura, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
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DECLARATION 2 - PUBLICATIONS

Author's contribution to publications that form part of this thesis:

Publication 1 (Chapter 2)

M.E. Griffiths, M.J. Lawes and Z. Tsvuura. Understorey gaps influence regeneration dynamics in subtropical coastal dune forest

Author contributions:

MEG and ZT collected data. MEG wrote the paper. MJL and ZT contributed valuable comments to the manuscript.

Publication 2 (Chapter 3)

Z. Tsvuura, M.E. Griffiths and M. J. Lawes. The effect of herbaceous understory cover on fruit removal and seedling survival in coastal dune forest trees in South Africa

Author contributions:

ZT conceived paper with MEG and MJL. ZT collected data and wrote the paper. MEG and MJL contributed valuable comments to the manuscript.

Publication 3 (Chapter 4)

M. E. Griffiths, Z. Tsvuura, D. C. Franklin and M. J. Lawes. Pollination ecology of *Isoglossa woodii*, a long-lived synchronously monocarpic herb from coastal forests in South Africa

Author contributions:

MEG and ZT conceived paper with MJL. MEG and ZT collected and analysed data, and wrote paper. DCF and MJL contributed valuable comments to the manuscript.

Author’s contribution to unpublished chapters that form part of this thesis:

Chapter 5

Z. Tsvuura, M.E. Griffiths, R.M Gunton and M. J. Lawes. Predator satiation and recruitment in a mast fruiting monocarpic forest herb

Author contributions:

ZT conceived paper with MEG and MJL. ZT and RMG collected and analysed data. ZT wrote the paper. MEG, RMG and MJL contributed valuable comments to the manuscript.

Chapter 6

Z. Tsvuura, M.E. Griffiths and M. J. Lawes. Competitive effects of a dominant understorey herb *Isoglossa woodii* (Acanthaceae) on tree seedlings in a subtropical dune forest in South Africa

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Chapter 7

Z. Tsvuura, M.E. Griffiths, R.M. Gunton, P.J. Franks and M. J. Lawes. Photosynthetic responses and relative shade tolerance of coastal dune forest tree seedlings in response to a suppressive herb

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ZT conceived paper with MEG, PJF and MJL. ZT, MEG and RMG collected data. ZT wrote the paper. MEG, RMG, PJF and MJL contributed valuable comments to the manuscript.

Chapter 8

Z. Tsvuura, M.E. Griffiths, R.M Gunton and M. J. Lawes. The effects of *Isoglossa woodii* on woody seedling establishment in a coastal dune forest

Author contributions:

ZT conceived paper with MEG and MJL. All authors collected data. ZT wrote the paper. MEG, RMG and MJL contributed valuable comments to the manuscript.

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CHAPTER 1

INTRODUCTION

Background

In this thesis I investigate the effect of a pervasive understorey herb *Isoglossa woodii* on tree dynamics and composition in a coastal dune forest. In recent years the role of understorey vegetation as an ecosystem driver has received attention (George and Bazzaz 2003, Nilsson and Wardle 2005, Gaxiola et al. 2008), the principle being that understorey vegetation acts as an ecological filter, suppressing tree seedling regeneration and affecting belowground processes, and in so doing is a driver of forest community and ecosystem properties.

In addition, I investigate the evolutionary biology of *I. woodii*. The species has relatively long periods of vegetative growth that are followed by synchronized reproductive and mortality events. The ultimate advantages conferred on a species by synchronized reproduction have been the subject of many studies (Silvertown 1980, Waller 1993, Kelly 1994, Kelly and Sork 2002), and this is a feature of *I. woodii* that this study also seeks to unravel. The ultimate effects on forest dynamics is considered against this background, specifically how *I. woodii* affects forest regeneration and why the species is synchronously monocarpic.

Understorey dynamics and forest tree regeneration

Understorey plants are an important structural component of forest, and may play a vital role in regeneration of overstorey trees (Maguire and Forman 1983, George and Bazzaz 1999). Falling seed must pass through the understorey stratum before it reaches the forest floor. Should the seed germinate, the environment of the seedling is largely influenced by understorey vegetation. For example, in fern-dominated forest understorey, the fern stratum is an ecological filter that shapes the composition and organization of regeneration by influencing the emergence and establishment of tree species (Denslow et al. 1991, George and Bazzaz 2003, Nilsson and Wardle 2005). This understorey filter affects regeneration through selective influence on density, species composition, size structure and spatial distribution of seedlings.

The negative influence of the understorey on forest regeneration may operate through reduced irradiance on the forest floor. The magnitude of understorey reduction of light varies between forests, and ranges from < 2 % in many tropical forests to 10-12 % radiance at the forest floor (Nakashizuka 1987). The difference in the light levels passing through the understorey layer reflects differences in understorey density and architecture. Such reductions in availability of light may have important implications for growth, survivorship and diversity of seedlings. For example, the low light levels beneath *Rhododendron ponticum* (Ericaceae) dominated understorey thickets is a major cause of tree seedling mortality in the Killarney oakwoods of south western Ireland (Cross 1981). Poor seedling growth and survivorship under shaded conditions occur as a result of temporal carbon deficits associated with light levels below the compensation point of photosynthesis (Harper 1977). The effect of shade on growth and survivorship of plants varies between species (George and Bazzaz 1999a), and may also vary between size classes within a species, which may result from different compensation points of photosynthesis for individual seedlings.

The availability of light, however, is not the only variable affected by understorey dominants. Soil resources (e.g., cation concentrations, moisture and mycorrhizae) and soil processes (e.g., nitrogen mineralisation rate) may be reduced while allelopathy, pathogens, and seed and seedling predation may be more pervasive under subcanopy thickets relative to areas outside thickets (Augspurger 1984, Denslow et al. 1991, Molofsky and Fisher 1993, Lei et al. 2002, George and Bazzaz 2003, Slocum et al. 2004, Nilsson and Wardle 2005).

Shrubs and saplings of tree species can have similar effects to that of the herbaceous layer in negatively influencing tree regeneration in forests, while burial of seedlings under the leaf litter from overstorey or understorey dominants may also reduce regeneration (Lei et al. 2002, Slocum et al. 2004). However, understorey dominants do not completely curtail regeneration. Rather, they reduce it such that suppressed seedlings, saplings and small trees are present at low densities until a disturbance event occurs and results in elevated levels of the limiting resource. Seedlings and saplings growing under pre-disturbance resource levels constitute advanced regeneration. The species composition of advanced regeneration may be dependent on the shade tolerance of those species. Furthermore, reduced regeneration is not confined to the tree layer. Establishment and growth of understorey forbs and shrubs may also be suppressed (Veblen et al. 1977).

Disturbance such as treefall, fire or dieback of a monocarpic species suppressing regeneration (Janzen 1976, Struhsaker 1997, Gonzalez et al. 2002, Franklin and Bowman 2004) can alter forest regeneration through changes in light regimes. Higher intensity and duration of sunlight has been reported in canopy gaps (Chazdon and Fetcher 1984) as well as increased levels of soil moisture in the top-soil (Vitousek and Denslow 1986), which probably reflects a reduced transpirational surface. Post-disturbance elevated levels of these resources may stimulate rapid growth of early and late advanced regeneration. For example, *Fagus crenata*, a canopy dominant in Japanese temperate forests, regenerates after the death of *Sasa palmata*, a monocarpic understorey bamboo (Taylor and Qin 1988). Seedling success following gap formation is not, however, always realized. Recently formed canopy gaps may immediately become infested with pioneering vines, herbs and shrubs that may lower levels of tree regeneration in the gaps compared to that of the adjacent forest (Chapman et al. 1999). In addition, collapsing stems of both overstorey and understorey plants may smother some tree regeneration (Paul et al. 2004). Lawes and Chapman (2006) found that patterns of elephant herbivory had a greater effect than the herb stratum in suppressing regeneration in disturbed Afrotropical forest.

By reducing resource levels for tree regeneration, the herb cover can affect the structure and dynamics of the forest in several ways. Seedlings of shade-tolerant species can survive and grow in the low resource environment beneath tree canopies. In contrast, seedlings of shade-intolerant species may survive and grow, albeit slowly and at low density, until a disturbance increases resource levels (Denslow 1987). Increases of resource levels following disturbance vary according to the magnitude and frequency of disturbance. For example, light levels reaching the floor of canopy gaps increase with an increase in gap size (Chazdon and Fetcher 1984), which may mean that some seedlings encounter a suite of optimum conditions after canopy disturbance. However, some canopy gaps are too small to promote advanced regeneration, and are filled in by lateral ingrowth from branches of neighbouring trees.

With canopy gap formation fairly constant from year to year in Neotropical forests (estimated at about 1 % per year: Denslow 1987), resource availability (in canopy gaps) and resource scarcity (under canopy) are expected to exist in continual flux so that tree regeneration occurs differently from site to site in the same forest. If disturbance regimes are fairly constant, the structure and dynamics of the forest will not change in the long term.

The dynamics of regeneration described above are based on data from tropical and temperate forests dominated by single-stemmed trees. The spatial association between regenerating and established plants is generally negative due to competition for resources, in contrast to the positive associations (e.g., shade provided to seedlings by canopies of adult plants in dry sites) that are frequently observed in arid and cold habitats (Tirado and Pugnaire 2003). Facilitative effects of established plants can occur even on productive, mesic sites. In a temperate lowland rainforest, seedling establishment sites are scarce on the forest floor while standing tree fern trunks provide opportunities for epiphytic regeneration (Gaxiola et al. 2008). In these forests, light levels are higher and herbivore pressure is lower than at the forest floor. Although facilitative interactions have received less attention in ecological research compared to competitive interactions, both interactions probably occur simultaneously (Holmgren et al. 1997).

The regeneration and persistence niches

Central to the evolution of life histories of plants is the trade-off between vegetative growth and seeding (Blarer and Doebeli 1996). The trade-off between growth and reproduction may be mediated by the amount of resources available. If resources are abundant it is more beneficial to the individual plant to allocate carbohydrate reserves to seeding, thereby maximizing its reproductive potential, whereas in resource-poor habitats, seeding may be too costly an option and plants trade off growth and reproduction for storage, resulting in fewer seeding bouts and longer periods of vegetative growth. For example, *Sideroxylon inerme* and *Pterocelastrus tricuspidatus* in

South Africa are multi-stemmed trees with few seedlings at forest sites of low productivity, but are single-stemmed and produce more seedlings at sites of higher productivity (Kruger et al. 1997). The trade-off between resprouting and seeding may be also dependent on the disturbance regime of the site. In ecosystems subject to periodic disturbance (e.g., fire, drought, wind-throw, herbivory), plants may persist by resprouting (Bellingham and Sparrow 2000, Nzunda et al. 2008), a strategy that entails allocating photosynthate to storage rather than to growth. In sprouter-dominated forests, trees are multi-stemmed and shorter, produce few seeds and seedlings (Bond and van Wilgen 1996, Bond and Midgley 2001, Nzunda et al. 2007a, c), have smaller seedbanks, have slower growth rates with lower seedling survivorship than forests dominated by nonsprouters (Kruger et al. 1997).

Plant strategies of reseeding and resprouting have been described as mechanisms constituting the regeneration and persistence niches, respectively. The regeneration niche concerns factors influencing the chances of successful juvenile recruitment from seed in different environments, while the persistence niche concerns factors that influence the chances of an adult plant retaining its space without regenerating from seed (Bond and Midgley 2003). The production and germination of seed initiate the regeneration of trees in forest. Harper (1977) regarded seedling germination as dependent on the occupation by seed of “safe sites”. A safe site offers a suite of conditions conducive to seed and seedling germination. This may include provision of the stimuli for breaking seed dormancy, conditions for germination to proceed, resources (water, oxygen) consumed during germination, and absence of hazards (e.g., predators, competitors, pathogens) to germination (Harper 1977).

For successful regeneration, several ecological and physiological factors and processes are constantly in operation, with some (e.g., optimum nutrient and moisture levels) promoting regeneration while others (e.g., drought, predators) curtailing it. The safe sites for seedlings can be extended to safe conditions for plant species regeneration. However, plant species exist in community assemblages, and the optimum conditions for regeneration may vary among species within the same habitats. For example, under a closed canopy seedlings of small-seeded or light-demanding species may be suppressed by deep litter and outcompeted for light by understorey dominants (Lei et al. 2002, Farris-Lopez et al. 2004). Under the same conditions of deep shade, survival of seedlings of large-seeded species may be independent of litter depth (Molofsky and Auguspurger 1992). Montgomery and Chazdon (2002) found that an understorey light gradient could alter the recruitment of shade-tolerant species in a tropical forest in northeastern Costa Rica. Even within species, tolerance ranges to shade may vary depending on local site conditions of nutrients (e.g., Grubb et al. 1996) and moisture, and between size classes (Canham 1989). While these studies show different responses of species to the understorey environment, the long term effects on community dynamics may not be important. Hubbell (2006) suggests that for shade tolerant species, strong dispersal and recruitment limitation prevent competitive exclusion, resulting in functionally equivalent species in the community.

Factors that curtail regeneration are the disturbances that result in multi-stemming and resprouting. With disturbance-mediated resprouting, plants fall into a continuum of responses that may diverge with life history stage of the plant (Hodgkinson 1998) rather than a simple sprouter-nonsprouter dichotomy (Bellingham and Sparrow 2000). Resprouting is therefore the mode of persistence of established plants (Bond and Midgley 2001), and has been receiving increasing attention in the recent past (e.g., Knox and Clarke 2005, Nzunda et al. 2007a, b, 2008).

In southern Africa, multi-stemmed trees dominate coastal dune forests, and the dynamics of tree species regeneration in these forests may be different from those of tropical rain forests that are dominated by single-stemmed trees.

The study species

Isoglossa woodii (C.B. Clarke) (Acanthaceae) is an understorey herb common in coastal dune forests of southern Africa. It also occurs 10 km or more from the coast in isolated patches of a few stems in a few square meters to many stems covering several hectares. Individual plants grow up to 3 or 4 m tall, and making up to 95 % of the understorey (Griffiths et al. 2007), the species dominates the forest floor.

After growing vegetatively for several years, *I. woodii* reproduces and dies synchronously. The periodicity of flowering cited in the literature varies from four to eight years (Wood and Evans 1899, Clarke 1901, Henkel et al. 1936, Moll 1972, Ward

1980). Other sources suggest as much as 10 years (e.g., the collector's notes on the original type specimen at John Bews Herbarium, Pietermaritzburg). In this study the periodicity of flowering was 7 years, which is consistent with Moll (1972). During the vegetative phase of growth, the species exhibits some limited clonal spread. Plants die within months of seeding and the seed germinates immediately (pers. obs.).

The lack of seedlings of canopy tree species beneath *I. woodii* stands suggests that this species competes for space, light and nutrients with the seedlings of woody species and may affect tree community succession, even arresting succession in dune forest. Understorey gaps (*sensu* Connell et al. 1997) created by the dieback of *I. woodii* may provide an opportunity for pulse recruitment of coastal dune forest trees (Griffiths et al. 2007). Released from competition with *I. woodii* cover during these periods of recruitment, germination and establishment rates of tree species may increase and these woody seedlings may grow to a height that will ensure their competitive advantage before *I. woodii* begins to regenerate.

The life cycle of *I. woodii* may hold the key to coastal dune forest dynamics. Since the herb likely suppresses the growth of tree seedlings, and the entry of new recruits into the population can only occur when *I. woodii* dies back, forest tree recruitment is predicted to be very closely tied to the life history strategy of *I. woodii*. However, the regularity of *I. woodii* dieback may not favour high tree species diversity because it selects for species whose physiology allows them to grow quickly and become well established during the periods of competitive release that occur during *I. woodii* dieback. Therefore, *I. woodii* causes infrequent recruitment opportunities that may lead to a convergence of tree life history traits, tree species composition and ultimately constrain forest diversity. On the other hand, limited recruitment periods caused by *I. woodii* may enhance forest diversity if the understorey gaps created during the dieback restrict the establishment of persistent, vegetatively reproducing species (i.e., resprouters) while providing opportunities for non-sprouting seed producing species to establish in the system.

Determining the nature of these putative interactions between *I. woodii* and forest tree regeneration dynamics is the main aim of this thesis, in addition to investigating why this herb evolved synchronous flowering. It has been suggested that synchronous flowering results in a higher seed set than can be consumed by predators (predator satiation hypothesis; Janzen 1976), that seedlings of masting species swamp other species' seedlings (competitor swamping hypothesis; Struhsaker 1997), and that mass flowering enhances outcrossing (outcrossing enhancement hypothesis; Janzen 1976). Also, the proximate cause or cue that causes the herb to shift from predominantly vegetative growth to reproduction has not been identified. A better understanding of the autecology and life history of *I. woodii* will contribute to our understanding of coastal dune forest plant dynamics.

Monocarpy

Monocarpic, or semelparous plants, reproduce once before they die. Plants exhibiting repeated reproduction throughout their lifetime are referred to as polycarpic or iteroparous. While monocarpy is a characteristic feature of annual plants (therophytes), some non-annual species are also monocarpic, with representatives in many families (Young and Augspurger 1991). A few species in the Acanthaceae and many species of herbaceous and tree-like bamboo (Poaceae) are monocarpic (Janzen 1976, Tweedie 1965, 1976). Perennial monocarps consist of species that are monocarpic by morphology (e.g. *Ensete* and *Corypha* (Palmae), *Spathelia* (Rutaceae)) and species that are monocarpic by adaptation (Simmonds 1980). The first group is made up of unbranched plants with a single growing tip that is converted into a terminal inflorescence. Post reproductive death of the terminal inflorescence precedes death of the whole plant because the single growing tip is no longer vegetative. In the second group (monocarps by adaptation), plants have multiple branches and die after fruiting as an evolutionary response to a selection pressure (e.g., many bamboos, *Tachigalia* (Leguminosae), several Acanthaceae). The discussions of monocarpy in this thesis will focus on the second group of monocarps by adaptation.

Monocarpy is best described for species of bamboo, within which life-spans range from less than a decade to more than a century (Janzen 1976). Most monocarpic bamboos flower after 15 to 60 years (see Janzen 1976, Table 1). In the monocarpic Acanthaceae, life-spans are also variable between species, ranging from 3 years to 16 years for

Isoglossa and *Strobilanthes* species respectively (Janzen 1976, Tweedie 1976, Sharma et al. 2008).

Evolution of monocarpy

The scattered occurrence of monocarpy across many plant families suggests that the phenomenon has evolved independently several times in different species (Simmonds 1980, Silvertown 1983, Young and Augspurger 1991), and has been the subject of many theoretical analyses (Gadgil and Bossert 1970, Schaffer and Schaffer 1979). These analyses assume that there is a trade-off between reproducing at the present time and reproducing in the future, or alternatively a trade-off between growth and reproduction. A plant that delays reproduction and uses its resources for vegetative growth until it has grown to large size may lose opportunities for reproduction and have lower fitness compared to an early maturing plant. For many species however, the probabilities of survival and of flowering increases with size (Metcalf et al. 2003, Table 1). When the plant eventually flowers, the question becomes whether the large size attained through trading off reproduction results in higher reproductive output and fitness. This is indeed the case with many plant species: fecundity increases with size (see Tables 2 and 3 in Metcalfe et al. 2003). The demographic patterns of high reproductive output and higher probabilities of survival and flowering with large size are similar in most monocarps. However, polycarpic plants closely related to these monocarps also have extended juvenile stages (Young and Augspurger 1991), which may diminish the value of long maturation periods as a condition for the evolution of monocarpy. Delayed maturation may be linked to the need for plants to acquire sufficient resources for reproduction in both monocarp and polycarp species, but the output per reproductive episode is higher for monocarps (Smith 1983, Young 1990). The higher reproductive output of monocarps may help explain the subsequent mortality that occurs post-reproduction. Monocarpic plants may transfer all resources to reproduction and retain none for maintenance of vegetative structures, which leads to the death of the plant. Because a long juvenile stage is required to attain resources sufficient for a massive bout of reproduction leading to death of the plant, extended maturation times are a necessary condition for the evolution of monocarpy.

If considerable reproductive effort is expended in producing flowers, then a large floral display may be important for attracting pollinators (Schaffer and Schaffer 1979) and enhancing outcrossing (Janzen 1976, Augspurger 1981, Burd 1994). The mechanisms of outcrossing enhancement with large floral displays should however, vary between species. In clonal species, neighbouring plants may be ramets of one genet, and pollen transfer between such ramets in self-compatible species may result in self-fertilization.

A long pre-reproductive period resulting in large floral displays may not be related to attracting pollinators at all. For example, the periods of vegetative growth for many species of bamboo extend for several decades (see Janzen 1976), yet the ensuing large floral display is not functionally related to attracting pollinators, as bamboo is wind pollinated. Furthermore, recent experimental evidence suggests that resources other than pollinators limit fruit set in these species (Udovic 1981, Udovic and Aker 1981), while some monocarpic species of *Agave* are clonally polycarpic (Paige and Witham 1987).

In productive habitats, plants attain sufficiently high levels of carbohydrate stores for repeated reproduction within shorter time periods, whereas in unproductive habitats, energy accumulation can take longer (Janzen 1974, Kelly and Sork 2002). Habitat productivity may be closely associated with the length of the pre-reproductive period, such that productive habitats favour the evolution of a shorter pre-reproductive period and a polycarpic life history strategy. Plants in unproductive environments should therefore tend towards supra-annual reproduction due to the longer time required to recover between high seed crops. If post-seeding adult mortality is high, supra-annual seeding in low productivity environments can take the form of monocarpy (Young 1990), which may be accompanied by dominance of the site by the species.

In heterogeneous environments exhibiting low temporal stability in juvenile survival, the occurrence of an occasional extreme season may result in juveniles completely failing to survive, which would be a lethal strategy for a monocarpic species (Taylor and Inouye 1985, Charlesworth 1994). For a polycarpic species, however, there is more than one opportunity for reproduction so that an occasional lethal season would not threaten the species. Monocarpy is therefore favoured in environments with little fluctuation in juvenile survival. In a similar manner, fluctuations in adult survival may mean that in certain years, a high proportion of the population fails to survive. For a species with high year-to-year fluctuations in adult survivorship, the optimal strategy would be a single reproductive episode leading to monocarpy (Young 1990).

Janzen (1976) suggests by the predator satiation hypothesis that monocarpy may also evolve in populations subject to high levels of seed predation. In this hypothesis the offspring of an individual plant have an increased chance of growing to reproductive maturity if the parent plant produces a large surfeit of seed resulting in satiation of local seed predators. In a masting population, seeds are produced in large numbers at supra-annual intervals, and it is the long intervals between seeding that starves specialist seed predators (the 'numerical response', *sensu* Kelly 1994), resulting in low predator populations that are swamped in mast years (the 'functional response', *sensu* Kelly 1994). For a monocarpic species, increasing the length of the juvenile phase when the plant accumulates resources for the single massive bout of reproduction increases the probability of starving predators during the 'lean' years.

Although several workers have described and discussed the evolution of the life history strategy of monocarps, there are few hypotheses about how monocarpy could have arisen. Janzen (1976) suggests that the starting point was a polycarpic species that grew for several years before flowering. On reproduction an individual puts all its reserves in one seed crop, thereby producing more seed than its conspecifics, which retain some energy reserves for future growth. A low density of seed predators near the "anomalous" individual ensures that predators are satiated. Should seedlings fail to establish that year, the anomalous individual would be eliminated and its contribution to the next generation nullified. This situation could occur in a tropical area where the rainy season is predictable resulting in high seedling survivorship and where seed predators are highly territorial such that years of food abundance do not lead to decomposition of the territory. The 'mutant' plants could further increase their seed crop by waiting a longer period before having a single seed crop. Meanwhile, the population that contains the mutants will have fewer individuals in seed in any one year, resulting in seed predators concentrating on the clumps in seed, which consequently fail to satiate the local predators. In successive generations, the mutant comes to constitute more of the population as it satiates local predators, while the parent genotype decreases. In the mutant genotype, variants with different time to reproductive maturity are selected against (see also Silvertown 1980).

Janzen's central hypothesis (predator satiation) appears to be supported by the conditions favouring a monocarpic life history strategy as opposed to polycarpy, particularly the long juvenile period and environments with stable juvenile survivorship. Features of his hypothesis on how monocarpy could have *started,* with predation being the main selective force, may be similar to those for other long-lived monocarps such as members of the Acanthaceae. Many tropical and subtropical species of the Acanthaceae are relatively long-lived, with synchronous reproduction and mortality events that occur in 3-16 year cycles (Tweedie 1976, Janzen 1976, Struhsaker 1997, Sharma et al. 2008). These monocarps usually dominate the understoreys of forests in which they occur (Tweedie 1976, Janzen 1976, Struhsaker 1997).

In this thesis, one relatively long-lived, understorey dominant subtropical species of the Acanthaceae is chosen for study. The thesis investigates the selective forces that have driven the evolution of a monocarpic life history strategy on the species. It also seeks to determine the effect of this dominant monocarp on forest tree diversity and dynamics. While answers to these questions may not all be available, the information from species with related life-forms and similar life history strategies may provide insights to where answers to these questions may lie.

Synchronous reproduction

Masting

Mast flowering or seeding is the synchronous and intermittent production of large flower or seed crops by a population of perennial plants (Kelly 1994). The phenomenon occurs more often in trees than shrubs (Silvertown 1980) and is related in occurrence to slowly maturing, long-lived and larger-seeded species (Waller 1993). Some monocarps, because of the synchronicity of reproduction before population-wide adult mortality (e.g. several species of bamboo and acanthaceous herbs and shrubs), are considered masting species (Janzen 1974, 1976). Janzen (1978) categorises masting into two distinct types: (i) synchronous reproduction by monocarpic species, cued by an endogenous physiologic calendar, and exemplified by some bamboo and acanthaceous species, and (ii) synchronous reproduction by polycarpic species. Several workers suggest that polycarpic masting may be triggered exogenously by a weather cue, such as a severe dry spell, drought, or a drop or rise in mean temperature (Janzen 1978, Ashton et al. 1988).

Kelly (1994) categorises masting into three types: strict masting, normal masting and putative masting. Strict masting occurs in species whose reproduction is highly synchronized and where bimodality in seed crop size between years is evident (that is, in some years seed are produced, in others no seed are produced). Synchronously flowering bamboo and acanthaceous species show strict masting. Normal masting occurs when individuals in a population are not perfectly synchronized such that seed output among years varies continuously, evidenced by overlap of the tails of the seeding distribution. Poorly synchronized monocarps with masting spread over several years, or through overlapping cohorts (e.g., some bamboo species: Janzen 1976, *Tachigalia versicolor*: Foster 1977), and most polycarps show normal masting. With putative masting, only environmental variation has been identified as an explanation for variation in seed output between years, and because of this, the rest of the discussion on synchronous reproduction will focus on strict and normal masting.

Hypotheses for synchronous reproduction

For strict masting species where the intermast interval is several-to-many years, the evolution of the masting habit can be explained in terms of the predator satiation hypothesis. Indeed, Janzen (1976) explained (i) a long pre-reproductive period, (ii) population level synchronous reproduction (masting) and (iii) perennial monocarpy of bamboos, in terms of predator satiation. Satiation of predators can be achieved with the first two features which constitute normal masting, but the role of death of the parent plants (monocarpy) cannot be explained in terms of predator satiation. Several studies (Janzen 1971, Silvertown 1980, Ashton et al. 1988, Kelly and Sullivan 1997) invoke predator satiation for polycarpic masting species. It is unlikely that the same evolutionary advantage of predator satiation can be applied to two life histories consisting of masting alone as opposed to masting followed by post-masting death. In addition, some workers (Gadgil and Prasad 1984, Keeley and Bond 1999) point out that for some long-lived species of monocarpic bamboo, the life cycle is too long (> 60 yr) for predator satiation to be the selective force. Moreover the predator satiation hypothesis is only explained in terms of consumers of seed, yet consumers of vegetative parts may play a bigger role in influencing the reproduction potential of the plant through constant removal of biomass.

Struhsaker (1997) reported similar rates of seed and seedling mortality between synchronously flowering acanthaceous monocarps and those of rainforest trees in a long-term study at Kibale Forest, Uganda, and argued that this does not support the predator satiation hypothesis. He proposed that synchronous monocarpy affords seedlings of the species bearing those traits a competitive advantage for resources over seedlings of other species. Struhsaker's hypothesis suggests that synchronous monocarpy may be related to interspecific competition and establishment of seedlings. When a monocarpic species is established, its dominance is maintained by synchronized mass reproduction and seedling establishment, which spatially excludes other species' seedlings beneath the parent plants. As the parent plants die off, their seedlings are released from competition, and due to the fast growth rates of semi-woody acanthaceous species they soon over-top and exclude woody species. This hypothesis is premised on adult individuals of the monocarpic species creating suitable conditions for seedlings when they die *en masse* after seeding. Post-reproductive mortality of adult plants facilitates the success of seedlings though reduced competition for resources. It is noteworthy that dying adult plants may not collapse immediately, but senescence of the individual plant occurs slowly and gradually, starting with leaf shedding through gradual decomposition of the standing tree to stem collapse when the branches have rotted and fallen off. Slow death and decomposition of adult plant parts may reduce smothering of seedlings at eventual collapse of the main stem.

The evolution of a strategy where adult plants die to facilitate success of their seedlings probably requires that seeds, and eventually seedlings, of an individual plant end up directly below the parent (Janzen 1976). The location of seed and seedlings directly below the parent plant is feasible for gravity or wind dispersed seed, for example, *Tachigalia versicolor* (Foster 1977), the bamboos *Dendrocalamus strictus* and *Bambusa arundinacea* (Gadgil and Prasad 1984) and *Isoglossa woodii*. In relatively dense, monospecific stands of a gravity-dispersed monocarp, nearby individuals are probably closely related (due to low distance seed dispersal), and death of the adult plants facilitates seedling recruitment, which does not invoke group selection, but rather kin selection (*sensu* Gadgil and Prasad 1984).

Synchronous reproduction has also been explained in terms of wind pollination. The hypothesis states that wind pollinated species can achieve greater pollination efficiency if the flowering effort is above average and highly synchronized (Kelly and Sork 2002). The main prediction of this hypothesis is that higher fruit set will be achieved if flower density is high; a result that has been obtained in a number of studies (Nilsson and Wastljung 1987, Norton and Kelly 1988, Smith et al. 1990).

The arguments for wind pollination can be applied to animal pollination too, in that large flower crops are beneficial through attracting more pollinators (Augspurger 1981). In this hypothesis, isolated plants should have a lower fruit/seed set. A second and related prediction is that plants that flower out of phase with the remainder of the population experience pollination difficulties that will select for flowering synchrony (Janzen 1978). Supporting evidence for the animal pollination hypothesis is scarce (Taylor and Inouye 1985). Kelly and Sork (2002) argue that animals are likely to be saturated by large flower crops, which negates the benefits of mass flowering. Klinkhammer et al. (1994) assert that a large floral display size may cause pollinator swamping of flowers resulting in pollen clogging. Pollinator swamping may increase pollen transfer within the plant and reduce pollen transfer to other plants. For species with high intra-plant flowering synchrony (large floral display size), pollinator movement within the plant may be higher than that to other plants, thereby reducing cross pollination (Augspurger 1980). A large floral display size can also be achieved with high interplant flowering synchrony, and pollinator movement between plants may depend on the density of pollinators. If pollinators occur at high density, as is apparent with social insects, interplant movement may occur, and for outbred species this may result in cross pollination.

Keeley and Bond (1999) proposed a fire cycle hypothesis as a better explanation for synchronous monocarpy in bamboos. The build-up of a widespread and synchronous post-reproductive fuel-load by monocarpic bamboos increases chances of occurrence of a wildfire. The dense, monospecific clumps of bamboo also increase the potential for lightning-ignited fires. This hypothesis is of less interest in coastal dune forests because of the absence of a fire history.

Reproductive synchronicity in Isoglossa woodii and tree regeneration

Monocarpic species whose reproduction is synchronous also show synchronous mortality of the adult plants after seeding. Dieback of a dominant component of a plant community should result in major changes to the structural and ecological functioning of that community. This can be compared to gap dynamics in tropical and temperate forests, where light levels reaching the forest floor increase with gap formation (Chazdon and Fetcher 1984).

If *Isoglossa woodii* acts as an ecological filter affecting the competitive environment of tree seedlings, then its dieback functions as an ecological release that could result in elevated levels of moisture (see Vitousek and Denslow 1986), nutrient and light availability, which would, in turn, result in increased growth, survivorship, species density and total density of seedlings. The availability of nutrients may be further increased by the gradual decomposition of *I. woodii* stems, leaves and roots. While there is no information on whether *I. woodii* produces allelopathic compounds, or that during its life cycle it promotes the proliferation of pathogens that negatively affect tree seedlings, any such effect would be diminished by dieback events.

Any advantages to tree seedlings associated with a dieback of *I. woodii* are not absolute, but will be curtailed by competition from emerging seedlings of *I. woodii*. Several workers (Foster 1977, Struhsaker 1997) suggest that the adult monocarpic plants die to facilitate the success of their seedlings. So the post-seeding mortality of a synchronous monocarp may provide a window of opportunity for forest tree regeneration that is shaped by the competitive relationship between seedlings of *I. woodii* and tree species. Although the intensity of the competitive association between seedlings may be less severe compared to that of adult *I. woodii* and tree seedlings, the fast growth rate of the semi-woody acanthaceous monocarp may result in tree seedlings being overtopped within a few months. For advanced regeneration of woody species however, the fast growth rates possible with ecological release may help ensure their successful establishment.

Aims and objectives

The primary aim of this study is to clarify the role of *Isoglossa woodii* in driving canopy tree species establishment and dynamics. A secondary aim is to determine the reproductive biology of *I. woodii,* and the selective forces that may have shaped it. The objectives of this study are:

1. To investigate the ecological and environmental correlates of the distribution of *Isoglossa woodii* in dune forest.
2. To investigate the roles of outcrossing and predator satiation as evolutionary advantages of synchronous monocarpy in *Isoglossa woodii*.
3. To determine the ecological effects of the extensive cover and putative recruitment window caused by *Isoglossa woodii* on coastal dune forest tree seedling dynamics and diversity.

Study sites

The study was carried out mainly at Cape Vidal (28°05'32"S, 32°33'40"E) in the iSimangaliso (formerly Greater St Lucia) Wetland Park in northern KwaZulu-Natal Province of South Africa. Mean annual rainfall ranges from 900-1200 mm spread evenly throughout the year (Tinley 1985, Schulze et al. 1997). In drought years plants experience severe moisture stress between July and August, when windy conditions prevail. Mean annual minimum and maximum temperature is 17.8 °C in July and 25.6 °C in January respectively. The topography is made up of low to high forested sand dunes and valleys that are longitudinal and near parallel to the coastline. The soil is a loose unconsolidated fine to medium-grained sand with moderate levels of organic matter.

The dune forest at Cape Vidal forms part of a narrow belt (0.1 - 4km wide) of contiguous natural forest that extends for some 240km along the south eastern coast of South Africa (Tinley 1985). The tree layer includes individuals of *Diospyros natalensis* (Ebenaceae), *Drypetes natalensis* (Euphorbiaceae), *Mimusops caffra* (Sapotaceae), and *Sideroxylon inerme* (Sapotaceae) (Moll 1972, Nzunda et al. 2007a, c). The understorey is largely comprised of *I. woodii*, which shows evidence of heavy browsing in some parts of the forest. Large mammalian herbivores include black rhino (*Diceros bicornis*), white rhino (*Ceratotherium simum*), hippopotamus (*Hippopotamus amphibious*), kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus scriptus*), red duiker (*Cephalophus natalensis*) and bush pig (*Potamochoerus porcus*).

Some minor components of the study were carried out at Mapelane Nature Reserve (28˚24'32"S, 32˚25'17"E), also in the iSimangaliso Park. The climate, topography, soil conditions and vegetation at Mapelane appear to be similar to that at Cape Vidal (see Venter 1976) except that the *I. woodii* population, unlike that at Cape Vidal, did not flower in 2007-2008 and probably follows a different reproductive cycle. Both species of rhino are absent at Mapelane although hippo and the smaller antelopes are present.

Outline of the thesis

The first chapter of the thesis describes the monocarpic life history strategy of *Isoglossa woodii*, and discusses how this life history could have evolved. The dominance of *I. woodii* in the understorey is described, and the ecological significance of this dominance and monocarpy with respect to forest tree regeneration in coastal dune forest is discussed. A brief outline of the regeneration and persistence niches of plants is provided, with emphasis on the trade-offs that an individual plant makes in terms of continued vegetative growth versus reproduction.

In the second chapter, the ecological correlates of the distribution of *I. woodii* in coastal dune forest are presented. Comparisons of soil fertility status and plant communities in open understorey and *I. woodii* understorey are made. The quantity of light (photosynthetically active radiation) that reaches the forest floor under *I. woodii* thickets and in areas devoid of *I. woodii* thickets is analysed and discussed. The effect of *I. woodii* cover on predation of seed and seedlings of tree species is investigated in Chapter Three.

Chapter Four describes the reproductive ecology and pollination biology of the species, and investigates the evolutionary advantages of synchronized reproduction in *I. woodii*. The breeding system and success rate of outcrossing in *I. woodii* are determined during a mass flowering event.

The predator satiation hypothesis is tested as an evolutionary explanation for synchronous reproduction in *I. woodii* in Chapter Five. *Isoglossa woodii* cover is extensive in the forest but it is absent from some patches, thereby forming natural understorey gaps. Results of an establishment experiment of the species in these gaps are also presented.

Isoglossa woodii may compete with seedlings of tree species for above- and below ground resources. In Chapter Six I report on a competition experiment between *I. woodii* and tree seedlings from four species (two singlestemmed species and two multistemmed sprouting species), which has important implications for the ecological filter model presented in Chapters Two and Eight.

Chapter Seven focuses on shade tolerance and photosynthetic response of some forest species at the seedling stage while Chapter Eight examines the broader ecological influence of *I. woodii* as an understorey dominant on tree diversity and dynamics. Seedling success is measured in areas where *I. woodii* naturally occurs versus areas cleared of *I. woodii*, and herbivory versus herbivory-exclusion treatments are nested within these broad categories.

Chapter Nine summarizes and integrates the several aspects of the study, and suggests directions for future comparative and complementary studies.

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CHAPTER 2

UNDERSTOREY GAPS INFLUENCE REGENERATION DYNAMICS IN SUBTROPICAL COASTAL DUNE FOREST

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Abstract

Dominant understorey species influence forest dynamics by preventing tree regeneration at the seedling stage. We examined factors driving the spatial distribution of the monocarpic species *Isoglossa woodii*, a dominant understorey herb in coastal dune forests, and the effect that its cover has on forest regeneration. We used line transects to quantify the area of the forest understorey with *I. woodii* cover and with gaps in the cover. Paired experimental plots were established in semi-permanent understorey gaps with *I. woodii* naturally absent and in adjacent areas with *I. woodii* present to compare plant community composition, soil, and light availability between the two habitats. *Isoglossa woodii* was widespread, covering 65-95% of the understorey, while gaps covered the remaining 5-35% of the area. The spatial distribution of this species was strongly related to tree canopy structure, with *I. woodii* excluded from sites with dense tree cover. Seedling establishment was inhibited by low light availability (<1% of PAR) beneath *I. woodii*. When present, *I. woodii* reduced the density and species richness of tree seedlings. The tree seedling community beneath *I. woodii* represented a subset of the seedling community in gaps. Some species that were found in gaps did not occur beneath *I. woodii* at all. There were no significant differences between the sapling and canopy tree communities in areas with *I. woodii* gaps and cover. In the coastal dune forest system, seedling survival under *I. woodii* is dependent on a species' shade tolerance, its ability to grow quickly during *I. woodii* dieback, and/or the capacity to regenerate by re-sprouting and multi-stemming. We propose a general conceptual model of forest regeneration dynamics in which the abundant understorey species, *I. woodii*, limits local tree seedling establishment and survival but gaps in the understorey maintain tree species diversity on a landscape scale.

Key words: ecological filter, herb stratum, plant community structure, spatial distribution, tree seedling establishment

Introduction

Understorey plant species have a profound impact on regeneration dynamics in forests. The presence of subcanopy vegetation can inhibit the establishment and growth of tree seedlings, as has been shown in rainforest in Costa Rica (Denslow et al. 1991), *Nothofagus* forests in Chile (Veblen 1982; González et al. 2002), Appalachian forests in the southeastern United States (Monk et al. 1985; Clinton and Boring 1994; Baker and Van Lear 1998; Nilsen et al. 2001), and hardwood forests of the northeastern United States (George and Bazzaz 2003). This inhibition can be related to aboveground processes, such as facilitating the transfer of insects or pathogens from understorey plants to seedlings (Denslow et al. 1991) or reducing the amount of light reaching the forest floor (Clinton and Boring 1994; Nilsen et al. 2001). The negative effects of subcanopy vegetation on seedlings are also attributed to belowground changes in soil fertility and pH (Nilsen et al. 2001), a reduction in soil moisture (Nilsen et al. 2001), and the production of allelopathic compounds (Nilsen et al. 1999).

Considering the potential of understorey species to restrict tree seedling establishment, gaps in the understorey cover may play a key role in forest regeneration. We have been studying this dynamic in subtropical coastal dune forests in South Africa. The understorey of this forest type is dominated by the native herb *Isoglossa woodii* C.B. Clarke (Acanthaceae) (von Maltitz et al. 2003), which forms dense monospecific stands that often exceed 2 m in height. We hypothesize that shading by this species prevents the growth of tree seedlings and inhibits tree regeneration in subtropical coastal dune forests under *I. woodii* cover. However, we have observed natural semi-permanent gaps in the understorey cover of *I. woodii*. Similar to gaps in the subcanopy layer (Connell et al. 1997), these gaps in the herb understorey may promote the growth of light-demanding woody species and maintain tree species diversity in coastal dune forests on a landscape scale.

In addition, the monocarpic life history strategy of *I. woodii* is likely to further influence coastal dune forest dynamics. Individuals of this species reproduce synchronously every 4-7 years and then die back (Van Steenis 1978), resulting in significant temporal variability in the physical structure of the forest understorey. It has been proposed that tree recruitment might be limited to the dieback period when seedlings are released from competition with *I. woodii* (Midgley 2000). This temporal variation may also serve to maintain species diversity in coastal dune forests over the long term.

Understanding the factors influencing the establishment and spatial distribution of *I. woodii* is essential for determining what controls coastal dune forest tree regeneration and to answer the wider question of how understorey cover influences forest dynamics. With that broader view in mind, the aims of this study were to measure the extent of *I. woodii* gaps and cover in the understorey of coastal dune forests and to investigate the association of *I. woodii* distribution with environmental factors such as topography, proximity to the ocean, soil fertility, extant canopy tree community structure and composition, and light availability. Finally, we examined the relationship of the herb understorey to seedling, sapling, and canopy tree density, species richness, and community composition in experimental paired plots that were established in areas with natural *I. woodii* gaps and *I. woodii* cover.

Methods

FIELD SITES

The study was conducted between February and August 2004 in the subtropical coastal dune forest at Cape Vidal, in the Greater St. Lucia Wetland Park, KwaZulu-Natal, South

Africa (28˚16' S, 32˚29' E). Mean annual precipitation at Cape Vidal is ~ 1200 mm and mean annual temperature is 20-22 ˚C (Schulze et al. 1997). The dominant canopy tree species in this forest include *Diospyros natalensis* (Harv.) Brenan, *D. inhacaensis* F.White, *Dovyalis longispina* (Harv.) Warb., *Euclea racemosa* Murray, *Drypetes natalensis* (Harv.) Hutch., *Sideroxylon inerme* L. and *Mimusops caffra* E.Mey. ex A.DC. (von Maltitz et al. 2003), which grow to form a canopy of 15 m or less. The understorey is dominated by *I. woodii* but there are occasional patches with no *I. woodii* cover (termed "gaps" for this study). We assumed that all *I. woodii* plants in the understorey were even-aged because this species is monocarpic and synchronous flowering, mass dieback, and regeneration from seed last occurred in the study area in 2000 (pers. obs.).

At Cape Vidal, transect and paired experimental plot sampling methods were used to determine the spatial patterns in *I. woodii* distribution and its putative effects on tree regeneration in coastal dune forests.

TRANSECT SAMPLING

Twelve line transects were set up to run perpendicular to the long axis, or crest, of the dune series and coastline. Transects were placed randomly with starting points at least 100 m apart on the crest of the primary dune. Transects ran inland from the primary dune crest, ranging in length from 115-400 m (mean ± 1 SD = 267.9 ± 72.2; Table 1), with transects less than 400 m long when impassable coastal thicket was encountered. At points 5 m apart along each transect, we placed a 1 m^2 sample quadrat and recorded topographic location (foredune, dune crest, backdune, dune slack), the presence or absence of *I. woodii*, the height and density of *I. woodii* (number of stems per 1 m^2), and height of the tree canopy. When a gap in *I. woodii* cover was encountered, we measured the gap area. Following the methods described by Runkle (1985), we calculated gap fraction by dividing the length of each transect with gaps in the *I. woodii* cover by the total length of the transect. While this sampling technique gives no information about the frequency with which gaps form in the understorey *I. woodii* layer, it is an appropriate method to give an unbiased estimate of spatial coverage (Battles et al. 1996).

PLANT COMMUNITIES IN UNDERSTOREY GAPS AND COVER

Gaps in the *I. woodii* cover are sparsely vegetated with tree seedlings and cover of herbaceous species such as *Laportea peduncularis* subsp. *latidens* Friis and the grasses *Dactyloctenium australe* Steud. and *Panicum maximum* Jacq. One hypothesis for the existence of such gaps is that the instability of the sandy dune substrate, particularly on steep slopes, reduces establishment probability of *I. woodii* and other understorey species. These gaps are semi-permanent in that, once created, they persist for the duration of the lifecycle of the surrounding *I. woodii*. Gaps are filled in from the edges by *I. woodii* regeneration from adventitious root suckers or from seed. However, as *I. woodii* seeds are very small and have poor dormancy, gaps that are not colonized within a few weeks of seeding persist until the species next reproduces (pers. obs.).

Using a stratified random sampling design, thirty pairs of experimental 10 × 10-m plots (area = 100 m^2) were established to compare gap areas that were naturally free of *I. woodii* cover (*Iw*-) with adjacent stands covered by *I. woodii* (*Iw*+) (N = 60 plots). This plot size was chosen because most *I. woodii* gaps fit this dimension (136.8 ± 188.5 m^2; mean gap area ± 1 SD). The vegetation of each plot was inventoried and all woody plants comprising the seedling, sapling and canopy layers were identified to the species level. Nomenclature was based on Coates Palgrave (2002). For species that could not be reliably identified in the field, voucher specimens were identified by the Bews Herbarium at the University of KwaZulu-Natal, Pietermaritzburg. Seedlings were defined as all woody plants <50 cm tall, saplings were considered to be any woody plants >50 cm tall with stems <10 cm dbh and canopy trees were defined as stems >10 cm dbh. For saplings and trees, we measured height and dbh of each individual throughout the plot. We also counted the number of stems per plant because the incidence of multi-stemming is high in this forest type (Kruger et al. 1997). Seedlings and *I. woodii* density were measured in five 1 m^2 subplots placed throughout each plot. To avoid statistical problems with pseudoreplication, we combined results from the five subplots to give one cumulative measure of seedling density, seedling species richness, and *I. woodii* stem density (per 5 m^2), as well as an average measure of seedling height and *I. woodii* height for each plot.

SOIL CONDITIONS IN THE UNDERSTOREY

Soil samples were taken to determine if some aspect of soil fertility was associated with *I. woodii* cover. To control for small-scale soil heterogeneity within the plots, ten soil sub-samples were taken throughout each plot and bulked for analyses. Soil cores were collected at two depths: 0-5 cm and 5-20 cm. The soil samples were analysed by the Soil Fertility and Analytical Services unit at Cedara Agricultural College, KwaZulu-Natal for the following fertility parameters: density (g/mL), phosphorous (P, mg/L), potassium (K, mg/L), calcium (Ca, mg/L), magnesium (Mg, mg/L), exchangeable acidity ($cmol_c/L$), total cations ($cmol_c/L$), humic acid saturation (%), pH (KCl), zinc (Zn, mg/L), manganese (Mn, mg/L) and copper (Cu, mg/L). For a subset of the paired plots, total soil nitrogen was assessed separately using the Kjeldahl method for extraction and then determining ammonium with a specific ion electrode (N, mg/kg).

LIGHT CONDITIONS IN THE UNDERSTOREY

We measured photosynthetically active radiation (PAR; $\mu mol.m^{-2}.s^{-1}$), which is the amount of light in the portion of the spectrum used by plants (400-700 nm) hitting a given area over a set time period. PAR was measured using point and line quantum sensors (Apogee Instruments, Utah, USA), attached to a data logger (CR200, Campbell Scientific, Utah, USA). We simultaneously measured total available PAR above the forest tree canopy, the PAR reaching the forest understorey with no *I. woodii* present and the PAR reaching the forest understorey below *I. woodii*. PAR was measured for five days each in six sites with different *I. woodii* stem densities from March 2004-March 2005. PAR above the canopy and at the forest floor was also measured for ten days each in 6 sites with no *I. woodii* cover and differing tree stem densities. For each site, a daily mean of % PAR reaching the understorey was calculated using readings from 1000-1400 h.

STATISTICAL ANALYSIS

The proportion of each transect with *I. woodii* gaps and *I. woodii* cover was calculated. Data on *I. woodii* stem density and height as well as tree canopy height at different topographic positions along each transect were analysed using MANOVA in SPSS version 11.5 (SPSS 2002). Differences in gap size at different topographic positions were analysed with ANOVA, while gap size associations with distance along transect were analysed using regressions. Where appropriate, data were $\log_{10}$ transformed for normality. Because the results of some regressions appeared to have a greater spread in points at one end of the axis than the other (e.g., the "envelope effect" described by Thomson *et al*. (1996)), we tested the assumption of homogeneity of variances using Levene tests.

Because *I. woodii* is hypothesized to act as a selective ecological filter of tree species recruits we used a combination of several metrics to examine differences in community species composition between *Iw*+ and *Iw*- experimental plots. Using plot pair as a blocking factor, blocked Multi-Response Permutation Procedures (MRBP) were performed in PC-ORD to test for differences in the community composition between *Iw*- and *Iw*+ plots. The MRBP calculates a test statistic (*T*) that describes the separation between tested groups, the probability (*P*) of a smaller or equal delta, and the effect size as determined by the chance-corrected within-group agreement (*A*). For the effect size metric, $A = 1$ when all items are identical within groups (delta = 0), $A = 0$ when heterogeneity within groups equals expectation by chance, and $A < 0$ with more heterogeneity within groups than expected by chance.

Turnover in seedling community composition among plots within the *Iw*- and *Iw*+ treatments was assessed with Whittaker's $ß_W$ diversity measures (Magurran 2004). An indicator species analysis was also conducted in PC-ORD to determine species-specific responses in seedling community composition between *Iw*- and *Iw*+ plots. A Monte Carlo test with 1000 permutations was used to determine whether the indicator values or species differed significantly between plot types. Species with a probability (*P*) of less than 0.05 were strongly influenced by the cover of *I. woodii*.

Stem density, species richness and height for the seedling, sapling and canopy layers were analysed with MANOVA using plot pair as a blocking factor. Soil parameters, excluding N, were also analysed with MANOVA using plot pair as a blocking factor; a separate analysis was run for each of the two sampling depths. N was analysed separately with a univariate ANOVA for each of the two sampling depths.

Additional regression analyses of data from *I. woodii* present plots were used to assess the relationships between *I. woodii* stem density, *I. woodii* height, seedling density, and seedling species richness. Using binary logistic regression we tested whether patterns in the extant vegetation (tree height and tree stem density) or edaphic factors (topsoil P, K, Ca, Mg, or pH) were related to the distribution of *I. woodii*. Nitrogen was not included in this analysis because it was only measured in a subset of the paired plots.

Results

SPATIAL DISTRIBUTION AND MORPHOLOGY OF UNDERSTOREY COVER

The proportion of transects covered by *I. woodii* ranged from 65 to 95%. Gaps were present in the *I. woodii* cover throughout the transects and the proportion of forest with *I. woodii* gaps ranged from 5-35%. The size of gaps in the *I. woodii* cover did not differ according to topographic position (ANOVA; $F_{3,54} = 2.16$, $P = 0.103$; data not shown), but gap size increased with distance from the primary dune crest ($r = 0.263$, d.f. = 56, $P = 0.046$; data not shown).

Isoglossa woodii plants of greater height grew at significantly lower stem densities ($r = -0.403$, d.f. = 521, $P < 0.001$; data not shown). *Isoglossa woodii* stem density ($F_{3,519} = 14.47$, $P < 0.001$) and height ($F_{3,519} = 48.84$, $P < 0.001$), and canopy tree height ($F_{3,519} = 8.71$, $P < 0.001$) all varied according to topographic position (MANOVA; Wilks' $\lambda = 0.73$, $F_{9,1258} = 19.44$, $P < 0.001$) (Fig. 1). *Isoglossa woodii* was shorter and more dense on the dune crest and taller and less dense in the dune slack. Canopy tree height was also shortest on the dune crest, but was tallest on the backdune.

PLANT COMMUNITIES IN UNDERSTOREY GAPS AND COVER

Seedling community composition differed between *Iw*- and *Iw*+ plots (MRBP; $T = -3.00$, $A = 0.006$, $P = 0.004$). Turnover in species community composition among *Iw*- plots (average Euclidean distance = 13.49, $ß_W = 3.95$) was greater than for *Iw*+ plots (average Euclidean distance = 12.85, $ß_W = 3.54$). A comparison of species composition revealed 33 seedling species present in *Iw*- plots, but only 23 species in *Iw*+ plots. Of these species, 13 were restricted to *Iw*- plots, 3 to *Iw*+ plots, and 20 were shared between the two plot types. An indicator species analysis found significantly fewer *Adenia gummifera* (Harv.) Harms (P = 0.011), *Erythrococca berberidea* Prain (P = 0.004), and *Grewia caffra* Meisn. (P = 0.042) seedlings in *Iw*+ plots. Together with the results for species turnover for a plot type, these data suggest that the seedling community beneath *I. woodii* cover is a predictable subset of the total seedling species pool in gaps.

Both seedling density and species richness were higher in *Iw*- plots as compared with *Iw*+ plots (MANOVA; Wilks' λ = 0.84, $F_{2,56}$ = 5.17, P = 0.009). Seedling density was reduced from 24.07 ± 8.87 (per 5 m^2, mean ± 1 SD) in *Iw*- plots to 17.83 ± 8.59 in *Iw*+ plots ($F_{1,57}$ = 7.52, P = 0.008), while seedling species richness was reduced from 6.67 ± 1.88 in *Iw*- plots to 4.97 ± 1.88 in *Iw*+ plots ($F_{1,57}$ = 9.65, P = 0.003). In *Iw*+ plots, the responses of both seedling density (r = -0.31, P = 0.017) and species richness (r = -0.38, P = 0.003) varied according to *I. woodii* stem density (Fig. 2).

There were no significant differences between the two plot types in either the sapling (MRBP; T = -0.09, A < 0.001, P = 0.450) or canopy tree communities (MRBP; T = -0.60, A = -0.001, P = 0.716). We also found no differences between plot types in height, diameter, stem density, species richness, number of multi-stemmed individuals, and cumulative basal area of the sapling (MANOVA; Wilks' λ = 0.87, $F_{9,49}$ = 0.82, P = 0.601) and canopy tree layers (MANOVA; Wilks' λ = 0.85, $F_{10,48}$ = 0.86, P = 0.575). However, there were differences in canopy tree stem density and height that were associated with topography. Experimental plots situated on the dune crest had higher canopy tree stem densities (blocked ANOVA; $F_{3,55}$ = 4.67, P = 0.006) and lower canopy tree heights (blocked ANOVA; $F_{3,55}$ = 3.78, P = 0.015) than experimental plots in other topographic positions (data not shown).

SOIL AND LIGHT CONDITIONS IN THE UNDERSTOREY

Isoglossa woodii cover was not associated with soil density, P, K, Ca, Mg, exchangeable acidity, total cations, humic acid, pH, Zn, Mn, and Cu (MANOVA; 0-5 cm Wilks' λ = 0.86, $F_{12,47}$ = 0.64, P = 0.800; 5-20 cm Wilks' λ = 0.89, $F_{12,47}$ = 0.49, P = 0.913). There was also no association between *I. woodii* cover and N (ANOVA; 0-5 cm $F_{1,17}$ = 0.16, P

= 0.695; 5-20 cm $F_{1,18} = 0.07$, $P = 0.795$). No measured aspect of soil differed between *Iw*- and *Iw*+ plots (data not shown).

Isoglossa woodii cover reduced light availability at the forest floor (Fig. 3). Light levels were significantly reduced in *Iw*+ areas ($F_{1,51} = 67.64$, $P < 0.001$). We also found that the % available PAR at the forest floor in *Iw*- areas varied according to the canopy tree stem density ($r = -0.43$, d.f. = 59, $P < 0.001$). PAR was lower in areas with denser canopy tree growth (data not shown). However, we found no effect of canopy tree stem density on % available PAR in the presence of *I. woodii* ($r = 0.05$, d.f = 59, $P = 0.50$; data not shown). This indicates that *I. woodii* is a stronger filter of light than canopy tree cover. Regardless of canopy tree density, the presence of *I. woodii* will significantly reduce the PAR available to tree seedlings.

A binary logistic regression model of the ecological factors that potentially determine local distribution of *I. woodii* demonstrated that the height of canopy trees and soil P, K, Ca, Mg, and pH had no predictive power, but canopy tree density did (Table 1). Dense tree cover shades out *I. woodii*, as demonstrated by the fact that gaps in *I. woodii* cover were significantly associated with high canopy tree density. The odds of *I. woodii* occurring in a plot declined by 8.3% for each additional tree stem added in the model.

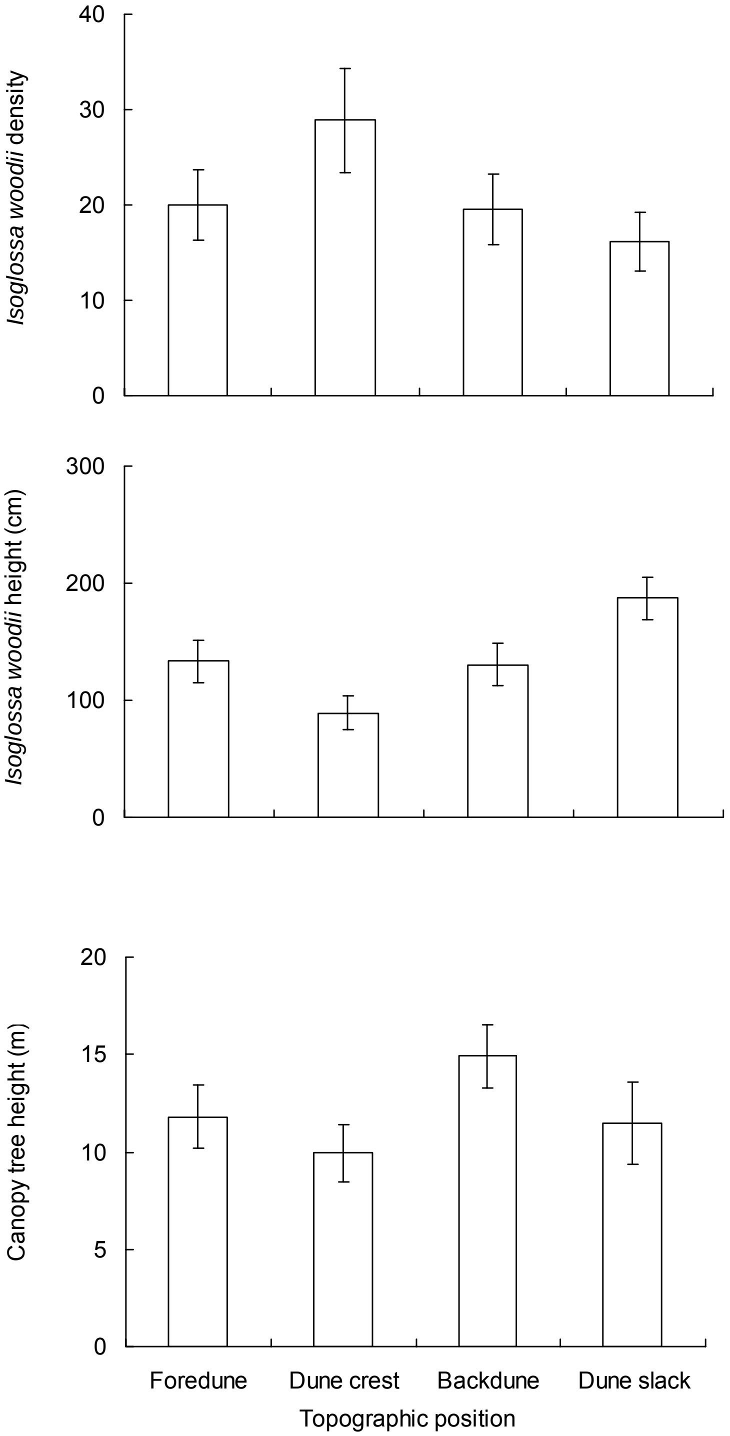

Fig. 1. Mean (± 1 SE) *I. woodii* stem density m^{-2}, *I. woodii* height, and canopy tree height in different topographic positions along line transects.

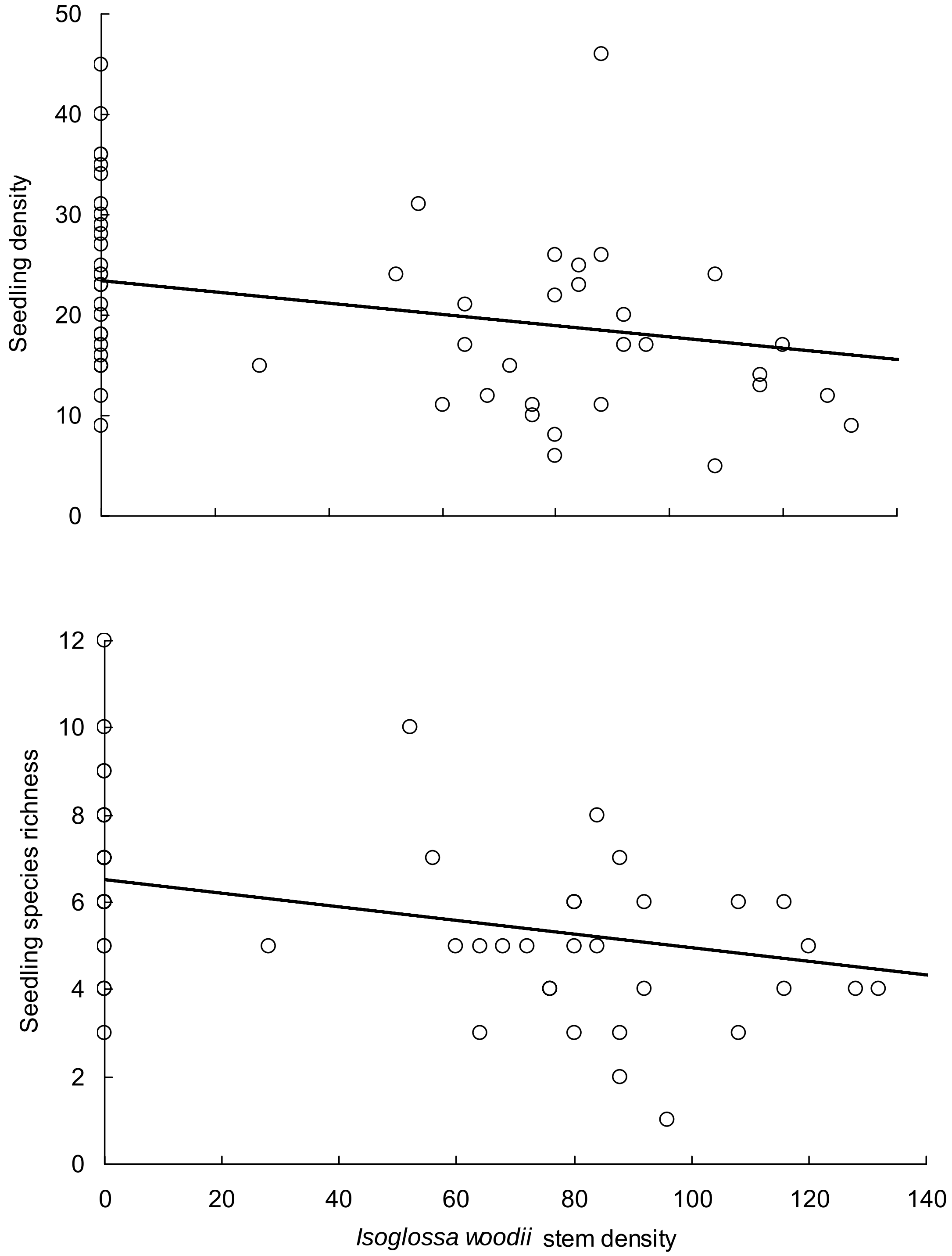

Fig. 2. Relationship of seedling density and species richness to *I. woodii* stem density per 5 m^2.

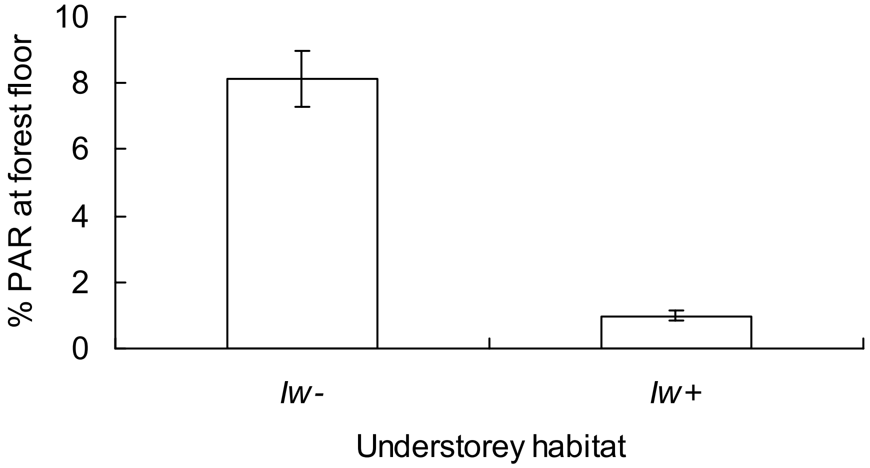

Fig. 3. Mean % available PAR (± 1 SE) at the forest floor in natural gaps with no *I. woodii* cover (*Iw*-) and areas with *I. woodi* cover (*Iw*+).

Table 1. Binary logistic regression model of *I. woodii* absence and presence in response to canopy tree height, canopy tree stem density, and topsoil P, K, Ca, Mg, and pH.

Variable	*B*	SE	Wald	d.f.	*P*
Tree height	0.090	0.075	1.445	1	0.229
Tree stem density	-0.103	0.048	4.655	1	0.031
P	-0.044	0.050	0.777	1	0.378
K	0.004	0.012	0.118	1	0.731
Ca	-0.001	0.001	1.420	1	0.233
Mg	0.007	0.007	1.196	1	0.274
pH	0.099	0.269	0.134	1	0.714

Discussion

There are many different abiotic and biotic processes that influence the abundance and distribution of a species, including soil fertility, topography, and light availability. In turn, the presence of a dominant species can profoundly affect the plants with which it coexists. Interspecific competition from a dense understorey stratum has been demonstrated to affect forest dynamics, both spatially and temporally (Veblen 1982; Monk et al. 1985; Clinton and Boring 1994; Baker and Van Lear 1998; Widmer 1998; Nilsen et al. 2001; González et al. 2002; George and Bazzaz 2003). *Isoglossa woodii* is indisputably the dominant understorey component in subtropical coastal dune forests. We documented semi-permanent gaps covering less than 35% of the area at Cape Vidal, with an increase in *I. woodii* gaps as one moves away from the ocean. This trend could be attributed to changes in the forest canopy structure, which becomes denser further inland.

Understorey species alter the physical structure of the community by creating a distinct understorey stratum beneath the tree canopy. Despite the fact that all plants were from the same cohort, we observed variation in the growth form of *I. woodii*. Our transect surveys demonstrate that strong intraspecific competition leads to self-thinning and reduced stem density in this species as height increases, which is similar to growth patterns in understorey bamboos (Widmer 1998). Furthermore, we found high variation in *I. woodii* height and stem density that corresponded with topographic position. *Isoglossa woodii* was shorter and grew more densely on dune crests and was taller and less dense in dune slacks. We attribute these differences to the biotic and abiotic conditions that exist in the different topographies. Experimental plots located on the dune crest had higher canopy tree stem densities, lower mean height, and lower PAR at the forest floor. Since canopy tree density affects the availability of PAR (Veblen 1982), this suggests that *I. woodii* distribution and growth is controlled by the light availability in the understorey resulting from forest canopy structure.

Acanthaceous herbs are known to limit forest regeneration (Chapman et al. 1999; Chapman and Chapman 2004; Paul et al. 2004). We found strong evidence that *I. woodii* inhibits the recruitment of trees, which supports results from other studies on dominant understorey species (Veblen 1982; Monk et al. 1985; Clinton and Boring 1994; Baker and Van Lear 1998; Nilsen et al. 2001; González et al. 2002; George and Bazzaz 2003). Seedling density was reduced in areas with *I. woodii* cover, as was seedling species richness, suggesting a species-specific effect of the herb on tree regeneration at the seedling stage. Indeed, some species were unable to grow in conditions created by *I. woodii*, yielding differences in seedling community composition between gaps and *I. woodii* cover. The clear separation we found in seedling communities between plots with and without *I. woodii* cover is commensurate with the ecological filter model proposed by George & Bazzaz (2003) in their study on the effect of ferns in northern hardwood forests.

Isoglossa woodii selectively filters or excludes tree seedling species in the understorey stratum. However, the filter effect is less obvious in the sapling and tree strata, either because (1) at *I. woodii* dieback canopy species establish in the understorey gaps and grow out into the canopy, or (2) differential mortality of seedlings caused by the filtering effect of *I. woodii* reduces the canopy tree community to a common composition. There was no evidence in this study to suggest that the canopy tree community was species poor compared to the seedling community. Thus, while *I. woodii* cover clearly inhibits seedling establishment and diversity in the short-term, periodic gaps in the herb cover, caused either by shading by canopy tree species or as part of the natural life cycle of the herb (i.e., dieback), may maintain tree species diversity over the long term in these forests (Connell et al. 1997).

One possible explanation for the observed differences in the seedling layer between *I. woodii* absent and present plots is that *I. woodii* modifies soil fertility, thereby limiting seedling growth. Previous studies have shown that dominant understorey species can alter pH and nutrients beneath their canopies (Nilsen et al. 2001), which could determine what seedlings are able to grow there. However, no measured aspect of soil fertility differed between *I. woodii* present and absent plots in this study, demonstrating that the edaphic properties we measured were not affected by this understorey species.

It has been proposed that seedling performance is primarily regulated by light availability (Pacala et al. 1996). We attribute the observed differences in seedling density and species richness to differences in available PAR, which was significantly reduced under *I. woodii*. During our sampling period, an average 1% of incident PAR penetrated below the *I. woodii* layer, which is less than the 2% PAR available beneath canopies of most forests (Canham et al. 1990). We also found no effect of canopy tree density on % available PAR under *I. woodii*, indicating that the species negates the effects of canopy trees on the understorey PAR environment. This degree of light inhibition by understorey cover and our interpretation that light is the factor responsible for limiting seedlings is consistent with findings by George & Bazzaz (2003).

Light availability also plays an important role in limiting where and how much *I. woodii* grows. We found a negative relationship between *I. woodii* height and canopy tree stem density and tree density best predicted where *I. woodii* grows. The limits to *I. woodii* distribution and growth can be attributed to less available PAR in areas with dense tree growth, as was found for bamboos in Costa Rica (Widmer 1998) and for the invasive weed *Tradescantia fluminensis* in native forests in New Zealand (Standish et al. 2001). If *I. woodii* colonizes open canopy sites with higher light availability, it might impede forest tree regeneration in areas that would ordinarily be important for the recruitment of less shade-tolerant seedlings (Widmer 1998).

THE ROLE OF *I. WOODII* IN DUNE FOREST TREE DYNAMICS

Our research suggests that the sparse growth pattern of canopy trees in coastal dune forests facilitates the dense growth of *I. woodii* in the understorey. In turn, *I. woodii* may maintain the structure in the canopy tree stratum by preventing seedling recruitment in areas with low canopy tree stem density. This could lead to the long-term persistence of *I. woodii* in the understorey layer. Such a model could explain why *I. woodii* is a dominant component of coastal dune forests and could also serve as a mechanism by which other understorey species such as *Rhododendron* spp. and *Kalmia* spp. in Appalachian forests (Monk et al. 1985; Lei et al. 2002), bamboos in Asian and Chilean forests (Veblen 1982; Ohkubo 1992; González et al. 2002), and other acanthaceous shrubs in central and west African forests (Chapman et al. 1999; Chapman and Chapman 2004; Paul et al. 2004) dominate the habitats in which they occur. The present study clearly demonstrates that *I. woodii* influences early regeneration. As a result, *I. woodii* may play a critical role in the dynamics of coastal dune forests.

Therefore, we hypothesize that *I. woodii* acts as an ecological filter by creating a light regime on the forest floor that favours tree species with shade-tolerant seedlings. Because of its monocarpic life history strategy, *I. woodii* also imposes temporal variation in the physical structure of these forests. Research in Chilean *Nothofagus* forests has documented the abundant regeneration of tree species after synchronous flowering by the bamboos *Chusquea culeou* and *C. tenuiflora* (Veblen 1982). Similarly, the dieback of *I. woodii* in the understorey of coastal dune forests may create a period of competitive release during which tree seedlings can establish and grow (Midgley, 2000).

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CHAPTER 3

THE EFFECT OF HERBACEOUS UNDERSTORY COVER ON FRUIT REMOVAL AND SEEDLING SURVIVAL IN COASTAL DUNE FOREST TREES IN SOUTH AFRICA

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ABSTRACT

Regeneration of forest canopy trees can be inhibited by understory thickets. We hypothesized that *Isoglossa woodii*, a large-leaved herbaceous plant, limits tree recruitment in subtropical coastal dune forests by providing habitat to fruit and seedling consumers. Using uncaged and caged treatments in *I. woodii* gaps and thickets, we found that frugivore and herbivore behavior is not influenced by *I. woodii*. We conclude that direct effects of *I. woodii* on postemergence processes in seedlings contribute to tree recruitment limitation.

Key words: Acanthaceae; herbivory; *Isoglossa woodii;* recruitment limitation; regeneration; seed predation.

Forest regeneration depends on the successful recruitment of seedlings. Seedling establishment is one of the strongest filters on tree recruitment (Clark *et al.* 1999), and has important consequences for forest regeneration dynamics (Hurtt & Pacala 1995). Therefore, any factors that contribute to tree seed and seedling mortality can have a profound impact on forest community composition, structure, and diversity at later stages.

In South Africa, coastal dune forest tree recruitment may be limited by *Isoglossa woodii* (Acanthaceae), a large-leaved herbaceous plant that forms a dense understory. Tree seedling number and diversity are lower in areas covered with *I. woodii* thickets (Griffiths *et al.* in press). This suggests that *I. woodii* limits tree recruitment, although the timing and mechanism of this influence is unclear. *Isoglossa woodii* could have direct effects on tree regeneration by limiting fruit production in parent trees through competition for moisture and nutrients, reducing fruit and seed dispersal by deflecting their fall into *I. woodii* thickets, preventing seed germination by allelopathy, or by limiting seedling establishment and growth through competition (Clark *et al.* 1998). Alternatively, *I. woodii* could have an indirect effect on tree recruitment by increasing herbivory on fruits and seedlings on the ground, as *I. woodii* cover provides habitat to frugivorous and herbivorous animals such as crested guineafowl (*Guttera pucherani*), red duiker (*Cephalophus natalensis*), bushbuck (*Tragelaphus scriptus*), bushpig *Potamochoerus larvatus*), and red bush squirrel (*Paraxerus palliates*) that occur in coastal dune forests.

The distribution of *I. woodii* in coastal dune forests is patchy, with natural gaps dispersed regularly throughout the understory. Predation risks in open areas can restrict foraging by vertebrate herbivores to areas with protective cover provided by understory vegetation (Seagle 1985, Longland & Price 1991, Schnurr *et al.* 2004). Red duikers, one of the most common herbivores in coastal dune forests, favor areas with dense understory vegetation (Bowland & Perrin 1995). Additional studies demonstrate that shrub cover increases herbivory of tree seeds (Wada 1993, Holl 2002) and seedlings (Abe *et al.* 2001, Lei *et al.* 2002). Seed and seedling removal could limit recruitment and have an inhibitory effect on tree regeneration (Schupp 1990, Wada 1993, Hulme 1996).

Furthermore, spatially nonrandom seed and seedling consumption may affect the distribution of saplings and adult trees (Janzen 1970, Connell 1971).

The aim of this study was to evaluate one possible mechanism by which *I. woodii* limits tree regeneration in coastal dune forest. We tested the hypothesis that *I. woodii* increases herbivory of tree fruits and seedlings in areas covered with *I. woodii* thicket. To address this, we measured fruit removal and seedling survival in herbivore exclosures in understory gaps and adjacent *I. woodii* thickets.

The study was conducted in subtropical coastal dune forest at Cape Vidal in the Greater St. Lucia Wetland Park in KwaZulu-Natal, South Africa (28°16' S, 32°29' E). These forests are characterized by a relatively short stature and a high density of multi-stemmed trees that result from the strong winds and unstable sandy substrate found in the coastal dune habitat. Canopy tree species include *Apodytes dimidiata* (Icacinaceae), *Diospyros natalensis* and *D. inhacaensis* (Ebenaceae), *Euclea racemosa* (Ebenaceae), *Mimusops caffra* (Sapotaceae), *Olea woodiana* (Oleaceae), *Sideroxylon inerme* (Sapotaceae), and *Teclea gerrardii* (Rutaceae), while subcanopy trees include *Allophylus natalensis* (Sapindaceae), *Dovyalis longispina* (Salicaceae), *Drypetes natalensis* (Putranjivaceae), *Erythroxylum emarginatum* (Erythroxylaceae), *Hymenocardia ulmoides* (Phyllanthaceae), *Ochna natalitia* (Ochnaceae), and *Tricalysia sonderiana* (Rubiaceae). The understory is dominated by *I. woodii* but there are occasional natural gaps in the cover by this species. Densities of herbivores such as red duiker and bushbuck are extremely high in the dune forests at Cape Vidal, contributing to regeneration failure in some subcommunities (Boyes 2004).

Experiments were carried out from June 2004 to September 2005, during which time there were no extreme climatic events. Using a stratified random sampling design, we established 30 pairs of experimental plots in two habitats: gaps in the understory where *I. woodii* was naturally absent (*I.w.*−) and areas directly adjacent to the gaps where *I. woodii* thickets were present (*I.w.*+). Experimental plots were all located below intact subtropical coastal dune forest canopy. Gaps used for the *I.w.*− treatments were standardized by size (with an average area of 200 m^2) because survival of seedlings can differ depending on gap size (Pearson *et al.* 2003). Thickets used for the *I.w.*+ treatments were standardized according to *I. woodii* height and stem density.

Fruit traps (150 × 150 × 50 mm) were constructed of 12 mm marine plywood. Screen mesh (2 × 2 mm) lined the base of the trap so that water did not accumulate and cause the fruits to decompose. Half of the traps were left with the tops open (uncaged treatment) and the other half were covered with 12 × 25 mm mesh (caged treatment). While the cages prevented fruit removal by birds, rodents, and game, they did not exclude insect herbivores such as ants and beetles. Fruit removal was measured in 25 experimental plots. At each plot, one uncaged and one caged fruit trap each were placed in the *I.w.*− and *I.w.*+ habitats. Fruit traps in the *I.w.*+ habitat were sited at least 5 m from the *I. woodii* gap to prevent edge effects. Pairs of uncaged and caged fruit traps were placed 1 m apart at equivalent distances from the gap edge.

We used fruits from *D. natalensis*, *D. longispina*, *E. emarginatum*, *H. ulmoides*, *M. caffra*, and *S. inerme*, which were chosen based on their prevalence in the forest and the availability of fruit during the study period. While there are no published data on fruit production in this forest type, we have observed high numbers of fruit drop and seedling germination in certain years, suggesting that some tree species mast fruit. The proximate cues that influence this interannual variation are unknown. The species we used in the experiment varied in fruit type, primary fruit consumers or dispersers, fruit size, and seed size, although all species had relatively small fruits and seeds and were limited to one or two seeds per fruit (Table 1). One species was tested at a time and each species was tested only once. Five fruits were placed in each trap and the remaining number of fruits was recorded after 3 wk.

Seedling herbivory experiments followed a similar design. Herbivore exclosures (300 × 300 × 150 mm) were constructed from 12 × 25 mm mesh. Survival of transplanted *D. natalensis* and *T. gerrardii* seedlings was monitored in 30 pairs of experimental plots, with each species tested at a different time. These species were used for the seedling herbivory experiments because they had abundant seedlings during the study. All seedlings were collected from Cape Vidal and were standardized for size and age. Five seedlings each were transplanted into an uncaged and caged area in the *I.w.*− and *I.w.*+ habitats in every plot. Seedlings that suffered transplant shock were replaced within 1 wk of transplanting. Seedling survival was monitored over a 6-wk period.

We analyzed fruit removal and seedling herbivory using blocked two-way MANOVA models in GenStat (GenStat Release 6.1, VSN International, Oxford, UK). Plot was used as a blocking factor so that paired *I.w.*− and *I.w.*+ treatments were considered together. For each species, the main effects of herbivore exclusion (uncaged vs. caged) and habitat (*I.w.*− vs. *I.w.*+) were tested, along with possible interactions. For fruit removal, analyses were based on number of fruits removed from each trap. Seedlings were analyzed according to percent survival data that were arcsine-square root transformed.

We found that fruit removal varied among species and treatments (Table 2; Fig. 1). Overall, the wind-dispersed species *H. ulmoides* had the lowest number of fruits removed, which is consistent with the fact that the fruit has no pulp to attract consumers. The number of fruits removed was significantly different between uncaged and caged fruit traps for *D. natalensis*, *D. longispina*, *E. emarginatum*, *H. ulmoides*, *M. caffra*, and *S. inerme* (Wilks' Lambda = 0.27, $F_{7,66} = 25.29$, $P < 0.001$). For all of these species except *S. inerme*, the number of fruits removed was lower in the fruit traps that were caged to exclude vertebrate herbivores. Total number of fruits removed differed significantly between *I.w.*− and *I.w.*+ habitats (Wilks' Lambda = 0.79, $F_{7,66} = 2.50$, $P = 0.024$), and there were significant interactions between caging treatment and habitat (Wilks' Lambda = 0.75, $F_{7,66} = 3.20$, $P = 0.006$), but these results were driven by a habitat effect on *M. caffra* and *S. inerme*. All other species showed no significant habitat or caging by habitat interactions.

Seedling survival also varied between species and among treatments (Table 2; Fig. 2). Overall, *D. natalensis* seedlings survived better than *T. gerrardii* seedlings. Herbivore exclusion did not affect seedling survival in either *D. natalensis* or *T. gerrardii* (Wilks' Lambda = 0.99, $F_{2,86} = 0.31$, $P = 0.734$). However, survival of seedlings was affected by habitat, with lower survival of *T. gerrardii* seedlings in *I.w.*+ habitats and higher survival in the *I.w.*− habitats (Wilks' Lambda = 0.93, $F_{2,86} = 3.17$, $P = 0.047$). There was no significant interactive effect between caging treatment and habitat (Wilks' Lambda = 0.99, $F_{2,86} = 0.07$, $P = 0.932$).

TABLE 1. *Fruit and seed traits for the coastal dune forest tree species used in fruit removal trials. Primary fruit consumer groups are included for all species except* H. ulmoides, *which is wind dispersed. Fruit and seed sizes are listed as length × diameter. Data from Coates Palgrave (2002).*

Species	Fruit type	Fruit consumer	Fruit size (mm)	Seed size (mm)	# seeds/fruit
Apodytes dimidiata	drupe	birds	4 × 3	2.5 × 2	1
Diospyros natalensis	berry	birds	12 × 6	7 × 3	1
Dovyalis longispina	berry	birds, mammals	15 × 15	9 × 6	2
Erythroxylum emarginatum	drupe	birds	13 × 7	11 × 6	1
Hymenocardia ulmoides	capsule	—	23 × 23	9 × 4	2
Mimusops caffra	berry	birds, mammals	20 × 15	15 × 10	1
Sideroxylon inerme	berry	birds, mammals	12 × 12	9 × 9	1

TABLE 2. *Species results from MANOVA tests of the influence of herbivore exclusion treatment, habitat created by absence or presence of* I. woodii, *and their interactive effect on fruit removal and seedling survival at the forest floor. As the models were blocked by plot, the plot stratum added an additional 24 df to the ANOVA models for the fruit removal experiments and an additional 29 df to the ANOVA models for the seedling survival experiments.*

	Cage			Habitat			Cage × Habitat		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Fruit removal									
Apodytes dimidiata	1,72	3.45	0.067	1,72	0.22	0.644	1,72	2.90	0.093
Diospyros natalensis	1,72	15.78	**<0.001**	1,72	0.07	0.798	1,72	1.64	0.204
Dovyalis longispina	1,72	76.72	**<0.001**	1,72	2.96	0.089	1,72	2.02	0.159
Erythroxylum emarginatum	1,72	33.30	**<0.001**	1,72	0.46	0.499	1,72	0.46	0.499
Hymenocardia ulmoides	1,72	9.17	**0.003**	1,72	0.37	0.547	1,72	1.02	0.316
Mimusops caffra	1,72	93.87	**<0.001**	1,72	5.42	**0.023**	1,72	12.46	**<0.001**
Sideroxylon inerme	1,72	22.23	**<0.001**	1,72	7.10	**0.010**	1,72	0.04	0.838
Seedling survival									
Diospyros natalensis	1,87	0.40	0.529	1,87	1.46	0.299	1,87	0.13	0.717
Teclea gerrardii	1,87	0.24	0.629	1,87	5.00	**0.028**	1,87	0.02	0.892

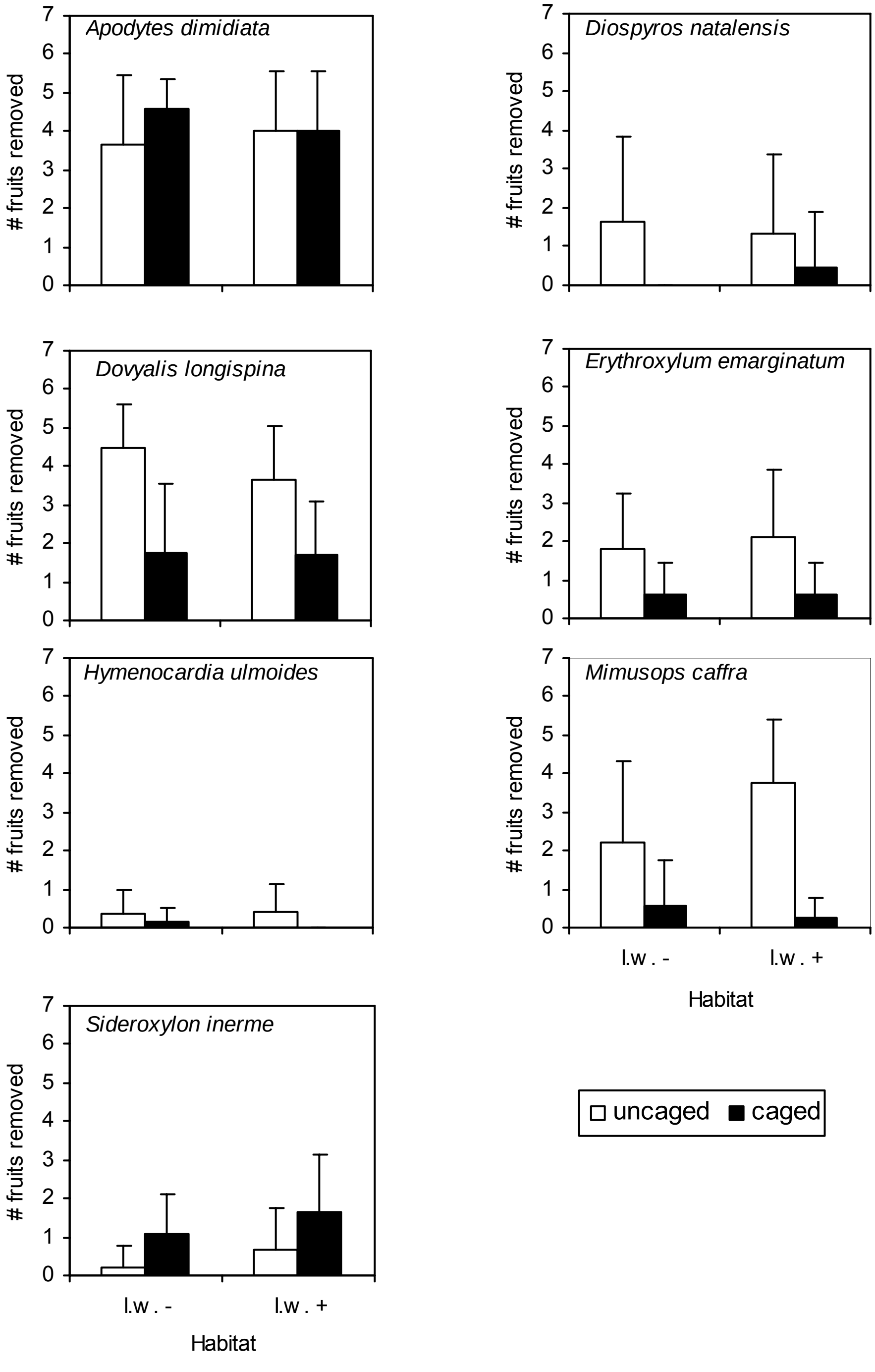

FIGURE 1. Influence of uncaged or caged treatments set in *I. woodii* gap (*I.w.*-) or *I. woodii* thickets (*I.w.*+) habitats on the number of fruits removed from fruit traps. Data are mean values ± 1 SD.

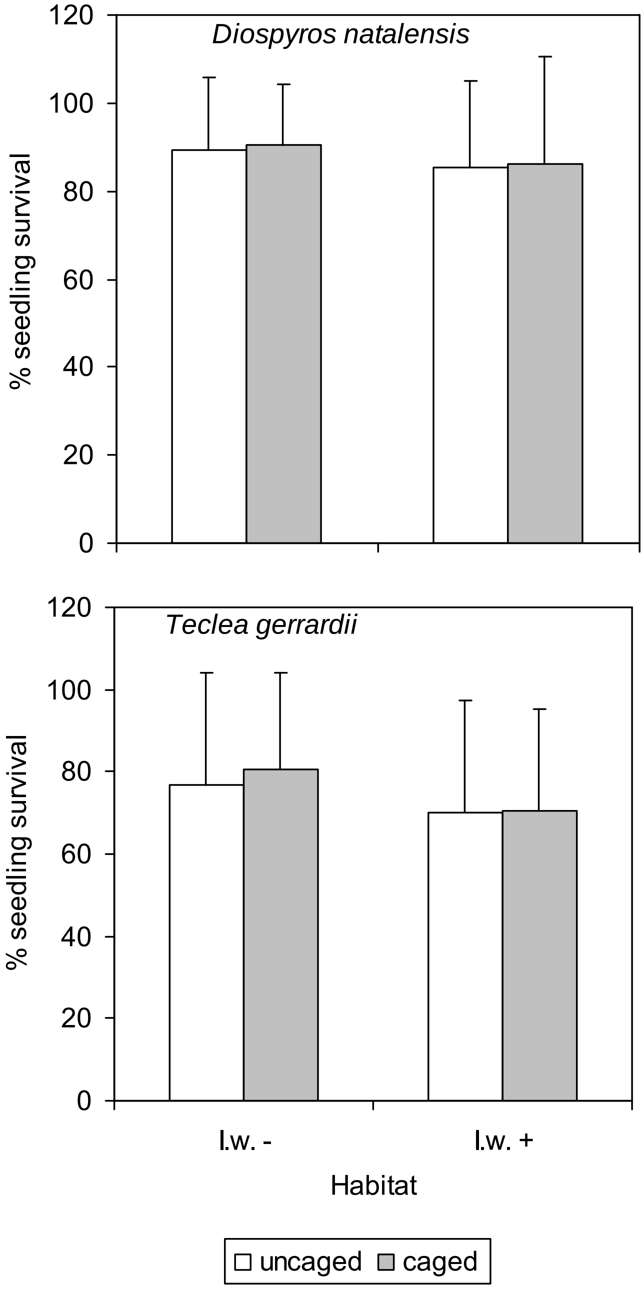

FIGURE 2. Influence of uncaged or caged treatments set in *I. woodii* gap (*I.w.*-) or *I. woodii* thickets (*I.w.*+) habitats on the seedling survival. Data are mean values ± 1 SD.

Studies by Wada (1993) and Abe *et al.* (2001) have demonstrated that fruits and seeds of tree species are consumed at higher rates under thickets of dwarf bamboo (*Sasa* spp.) compared to open understory. We found differential removal of fruits in caged and uncaged treatments but not between *I.w.*− and *I.w.*+ habitats. For all species except *S. inerme*, more fruits were removed from the uncaged traps. The removal of fruits from caged traps can be attributed to insects such as ants and beetles, whereas fruit removal from uncaged traps could be due to insects, birds, rodents, or game. Our results indicate that frugivores are present in the environment but their activity is not influenced by the absence or presence of *I. woodii*. The interspecific differences we observed suggest that some species are more palatable than others, which could result in lower seedling recruitment in species with palatable fruits and seeds.

Studies by Wada (1993), Abe *et al.* (2001), and Lei *et al.* (2002) have also demonstrated that herbivory on tree seedlings is higher in understory thickets. While herbivore exclosures have been shown to decrease seedling herbivory in such instances (Pearson *et al.* 2003), we found that caging had no effect on seedling survival in the tested species. However, *T. gerrardii* seedlings had lower survival in *I.w.*+ habitats, indicating that some factor other than herbivory limits seedlings in areas with *I. woodii*. This contradicts previous findings that seeds and seedlings are more influenced by mammalian herbivores than by site environmental conditions (Sork 1987).

We conclude that all of the tree species in our study are likely to experience some degree of recruitment limitation in coastal dune forests, either through fruit consumption by herbivores or by the suppression of seedlings in areas covered with *I. woodii*. Further research on the mechanisms driving seedling limitation in the coastal dune forest understory should focus on testing the hypotheses that *I. woodii* directly limits tree regeneration by deflecting the fall of fruit and seed into *I. woodii* thickets, preventing seed germination through allelopathic effects, or limiting seedling establishment and growth through competition for nutrients.

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CHAPTER 4

POLLINATION ECOLOGY OF *ISOGLOSSA WOODII,* A LONG-LIVED SYNCHRONOUSLY MONOCARPIC HERB FROM COASTAL FORESTS IN SOUTH AFRICA

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ABSTRACT

Synchronous monocarpy in long-lived plants is often associated with pollination by wind, in part because infrequent mass-flowering may satiate pollinators. Selfing in synchronous monocarps may provide reproductive assurance but conflict with the benefits of outcrossing, a key evolutionary driver of synchrony. We predicted that animal-pollinated species with synchronous flowering would have unspecialised flowers and attract abundant generalised pollinators, but predictions for selfing and outcrossing frequencies were not obvious. We examined the pollination biology of *Isoglossa woodii* (Acanthaceae), an insect-pollinated monocarpic herb that flowers synchronously at 4 – 7 year intervals. The most frequent visitor to *I. woodii* flowers was the African honey bee, *Apis mellifera adansonii*. Hand-pollination failed to enhance seed production, indicating that the pollinators were not saturated. No seed was set in the absence of pollinators. Seed set was similar among selfed and outcrossed flowers, demonstrating a geitonogamous mixed-mating strategy with no direct evidence of preferential outcrossing. Flowers contained four ovules but most fruits developed only one seed, raising the possibility that preferential outcrossing occurs by post-pollination processes. We argue that a number of the theoretical concerns about geitonogamous selfing as a form of reproductive assurance do not apply to a long-lived synchronous monocarp such as *I. woodii*.

Key words

Apis mellifera adansonii, breeding system, flowering synchrony, fruit and seed set, insect pollination, mixed mating system, nectar production, semelparity.

INTRODUCTION

Most plants flower at the same time as conspecifics, a process that facilitates outcrossing in many species (Augspurger 1981; Stephenson 1982; Burd 1994). Masting is a special case of synchronised flowering and fruiting in which synchrony occurs at supra-annual scales, one of several possible benefits of which may be an economy of scale provided by enhancement of pollination rates (Kelly & Sork 2002; Satake & Iwasa 2002). Synchronised monocarpy in long-lived plants is an even more extreme case of synchronised flowering because the interval between flowering may be exceptionally long (e.g. up to 120 years in some bamboos; Janzen 1976), because an entire generational cohort shares a single opportunity to reproduce successfully, and because there is little or no intra-individual variation in flowering intervals. Supra-annual flowering (= temporal aggregation) often also involves spatial aggregation and even monodominance (Kelly & Sork 2002), particularly in wind-pollinated plants where the benefits of cross-pollination may only be realised at short distances (Koenig & Ashley 2003; Davis *et al.* 2004; Ghazoul 2005).

Synchronised supra-annual flowering is more frequent amongst wind- than animal-pollinated plants because the benefits of masting may be negated by satiation of pollinators (Kelly & Sork 2002). Animal pollination can be quite effective among plants occurring at low density (Borges *et al.* 2003; Byrne *et al.* 2007), potentially eliminating the selective advantage of masting. Nevertheless, animal pollination is a feature of some masting plants (Pías & Guitián 2006), of a number of long-lived monocarps (Aker 1982;

Young 1982; Arizaga *et al.* 2000; Price *et al.* 2008), and of at least one long-lived synchronous monocarp (Sharma *et al.* 2008).

The evolution of synchronised supra-annual flowering may be driven by a range of processes of which facilitation of outcrossing is but one (Kelly 1994; Kelly & Sork 2002). Regardless, this life-history strategy has consequences for the pollination biology of the plants concerned. Amongst animal-pollinated species, supra-annual flowering renders the evolution of specialised pollination syndromes improbable, and the risk of pollinator satiation should favour simple floral structures to attract and exploit a range of generalist pollinators. Monocarps, especially those that are synchronous, are under even greater selection pressure to have a reproductive strategy that is robust to among-year variation in environmental and pollinator (and dispersal or establishment) conditions to avoid total reproductive failure. Trade-offs between display size and outcross rates (Albert *et al.* 2008) may be over-ridden by the imperative to maximise reproductive success in a single flowering event by the commitment of all available resources to it (Young & Augspurger 1991).

Although increased outcrossing is a potential advantage of synchronous flowering, we propose that a mixed mating strategy may be beneficial to synchronous monocarps because inbreeding can provide reproductive assurance. Notwithstanding the abundance of mixed mating strategies amongst plants with less specialised life histories, theoretical justification for reproductive assurance remains elusive and pathways for evolution of the strategy unclear (Goodwillie *et al.* 2005; Johnston *et al.* 2009). Mixed mating has been demonstrated in an animal-pollinated synchronous monocarp (Sharma *et al.* 2008), whereas the animal-pollinated masting tree *Sorbus aucuparia* is an obligate outcrosser (Pías & Guitián 2006). Ghazoul & Satake (2009) argued that production of unviable selfed seed may be beneficial to masting trees by facilitating satiation of seed predators. Synchronous monocarpy may optimise floral density within stands, a feature that may serve to increase outcrossing rates in plants with a mixed mating strategy (Karron *et al.* 1995).

In this study, we investigate the reproductive biology of *Isoglossa woodii*, a synchronously monocarpic herb that flowers in 4 – 7 year cycles (Van Steenis 1978). The objectives of the study are to: (1) examine the floral morphology and flowering phenology of *I. woodii* as it relates to the reproductive ecology of the species; (2) record floral visitors, observe their behaviour, and determine the mechanism for pollination in *I. woodii*; and (3) to determine the breeding system in this species. We interpret our findings in the context of the consequences of synchronous monocarpy for flowering in *I. woodii* and the debate about reproductive assurance in plants with a mixed mating strategy.

MATERIALS AND METHODS

Study species and site

Isoglossa woodii (Acanthaceae) is a robust, perennial herb growing to a height of 1 – 2 m. It is endemic to northeastern South Africa and southeastern Mozambique and is the dominant understorey plant in Indian Ocean coastal dune forests (Tinley 1985). This study was undertaken at Cape Vidal in the iSimangaliso Wetland Park, KwaZulu-Natal, South Africa (28˚16' S, 32˚29' E), where *I. woodii* is monodominant in the forest (Griffiths *et al.* 2007). The mean annual rainfall of this site is approximately 900 mm, spread evenly throughout the year, with a mean temperature of 21.5 °C (Schulze *et al.* 1997).

The species is synchronously monocarpic with flowering cycles of 4 – 7 years (Van Steenis 1978). Following flowering and subsequent death, regeneration is from seed that has no dormancy so that seedlings germinate in a single cohort (Z. Tsvuura, M.E. Griffiths and M.J. Lawes, unpublished data). At Cape Vidal, synchronous flowering of *I. woodii* occurred over 1000s of hectares in 2000 and again from April to September 2007. Observations and pollination experiments on *I. woodii* were carried out during 2007.

Isoglossa woodii plants expand laterally from adventitious root suckers (Griffiths *et al.* 2007), a feature that precludes ready identification of individuals. In this study we report some results on a per stem basis, selected stems being well-spaced to avoid sampling the same plant twice. Destructive sampling two years after mass-flowering demonstrated that plants may occupy an area of up to 1 – 1.5 m^2 with 1 – 10 stems per individual (M.J. Lawes, unpublished data).

Floral morphology

The inflorescences of *I. woodii* are arranged in racemes. Individual flowers are bilabiate, with white petals marked pink on the lower lip. The two stamens each have two anthers that are adpressed against the upper labium. The single, glabrous stigma sits between the stamens and the bi-locular ovary produces two ovules per locule. Single inflorescences on 50 randomly selected *I. woodii* stems were monitored throughout the flowering season to identify patterns of development and the number of flowers produced. Thirty flowers were examined and measured under a dissecting microscope with a calibrated ocular micrometer to estimate which insect visitors would be capable of pollen transfer. Measurements were made of: (1) flower length and breadth; (2) length of the floral tube, style and stamen; (3) distance between anther tips; and (4) the distance from the anthers to the lower labium (Fig. 1).

Pollen production was measured in one anther from each of three flowers on ten plants. Undehisced anthers were placed singly in microcentrifuge tubes containing 100 μL of HCl and crushed using a glass rod. The tubes were centrifuged at 5000 rpm for 10 min, after which the supernatant was removed and the pellet resuspended in 100 μL of a 3:1 solution of lactic acid and glycerol. After the solution had been vortexed for 30 s, three 1-μL subsamples were taken and the number of pollen grains counted under 10X magnification using a compound microscope.

To determine whether stigmas are receptive upon anthesis, we abscised styles (n = 30) of *I. woodii* at different stages of development (unopened buds and flowers open for 1 – 10 d), placed them in a 5 % hydrogen peroxide solution and monitored the rate of bubble formation (Mattson *et al.* 1974; Dafni 1992).

Nectaries are present at the base of the floral tubes in *I. woodii*. Diurnal patterns of nectar availability were assessed as the percentage of open *I. woodii* flowers in an inflorescence (one inflorescence per stem, n = 30 stems) that contained nectar in each of the early morning, at midday, and late in the afternoon.

The presence, volume and sugar concentration of nectar produced by *I. woodii* flowers over 24 h was assessed for ten stems protected by netting to exclude insect visitors. At the end of the 24 h, all open flowers were scored for the presence of nectar. Nectar was extracted from, and volumes measured for five flowers on each stem using a graduated 5 μL microcapillary tube. Because volumes were very small for the measurement of sugar concentration, the extracted nectar was pooled to form samples of 10 flowers and concentration measured using a Brix refractometer.

Fig. 1. *Isoglossa woodii* flowers. Ft = floral tube; la = lower anther; ll = lower labium; rs = receptive stigma; sta = stamen; sty = style; ua = upper anther; ul = upper labium.

Phenology

Phenological patterns were monitored at two spatio-temporal scales at Cape Vidal. Twenty stems were tagged and the number of inflorescences and flowers counted on 14 occasions during the 8.5 mo flowering period, including 5 occasions during the 2.5 mo initial flowering peak. Six 150 m transects were established and evaluated on 5 occasions, including 3 sampling sessions during the initial flowering peak. At 10 m intervals, a distance likely to exceed the spread of clonal individuals, the nearest stem within 2 m was scored for the presence of flowers. On the same stem we randomly selected three inflorescences and counted the number of flowers per inflorescence.

Floral visitors

The number and identity of potential pollinators visiting *I. woodii* flowers, and the number of flowers they visited, was documented in one 10 min survey in each of 80 randomly placed 0.25 × 0.25 m plots during the period of peak flowering activity. Observations were conducted between 9h40 and 14h00 and the temperature recorded along with the number of inflorescences.

In a separate exercise, the number of open flowers present and visited per inflorescence, and the distance traveled between inflorescences, was recorded for the primary floral visitor, *Apis mellifera adansonii*. For this purpose, 45 individuals were tracked for 5 min (n = 9) or until it was lost from sight if this occurred sooner (n = 36, 145 ± 15 s). As *A. m. adansonii* sometimes visited a flower more than once, the number of flowers visited sometimes exceeds the number present.

Ten *A. m. adansonii* were collected in sweep nets over flowering *I. woodii*. Pollen loads on the top of the head, upper body and lower body, and in the pollen sacs on the hind legs, were collected by swabbing with gelatine stained with fuchsin. Pollen loads were examined at 10X magnification using a compound microscope, grains being counted and identified as *I. woodii* or other species.

Breeding system

The efficacy of self- and cross-pollination in *I. woodii* was investigated on plants collected at Cape Vidal and grown in a greenhouse at the University of KwaZulu-Natal in Pietermaritzburg, South Africa. To exclude insects, plants were grown under shadecloth and the surface of greenhouse benches sprayed with an organophosphate insecticide. Three treatments were applied to flowers on each of six inflorescences on a total of seven plants: self-pollination by hand, cross-pollination by hand, and an unmanipulated control. For each flower, fruit set was assessed three weeks later.

The extent and rapidity of germination of pollen was compared on selfed and outcrossed flowers on two plants under the same greenhouse conditions as above. On each plant, six flowers each were selfed and six flowers cross-pollinated by hand and half the styles harvested after 24 h and 48 h, respectively. Styles were prepared for examination with a modification of the staining technique described by Martin (1959).

Styles were fixed in a formalin, alcohol and acetone mixture (8:1:1) for 24 h, washed with tap water and placed in NaOH solution for 24 h. The NaOH was subsequently removed by rinsing in tap water and the styles transferred to a 1 % solution of aniline blue for 8 h before mounting on miscroscope slides. Pollen tubes were examined at 10× magnification using an Olympus Provin AX70 fluorescent microscope at a wavelength of 365 nm. Pollen was considered to have germinated when the pollen tube extended down the length of the style.

A second experiment was carried out at Cape Vidal to determine whether *I. woodii* is pollen-limited in its natural habitat. We compared fruit and seed set in 25 pairs of plants in a randomized block design. Plants were paired in space and matched for size and flowering state, but were sufficiently separated (3 – 5 m) to preclude clonal connections. On one plant of each pair, all flowers on one inflorescence were cross-pollinated by hand to ensure that they received supplemental pollen. Another inflorescence on the same plant was unmanipulated and marked as a control (*internal control*). On the paired neighboring plant, one or two inflorescences were selected to serve as a second control (*external control*) to distinguish effects of pollen limitation from effects of resource shunting between inflorescences. Flowers open on day 1, 4 and 9 of the experiment were included, and fruit set counted as they developed over 6 weeks. For each inflorescence, a sub-sample of eight fruits (or all fruits if < 8 were available) were dissected to determine the number of seeds per fruit.

Statistical analysis

A self-compatibility index was calculated as the percentage of flowers that develop into fruits after self-pollination divided by the percentage of flowers that develop into fruits after cross-pollination (Lloyd 1968).

With the aim of assessing whether synchronised flowering may satiate the available pollinators whilst controlling for the effects of temperature on pollinator activity, the value of temperature and inflorescence density as predictors of bee visitation rates (flowers visited per inflorescence over 10 min, $\log_{10}[x+1]$ transformed) was assessed using simple and multiple least-squares linear regressions. Data from treatments in the greenhouse pollination experiment were pooled to the level of plants; the resulting randomized block design was analysed using Friedman's non-parametric ANOVA with post-hoc multiple comparisons following Zar (1984) as the control group had no variance. The response variables for the pollen limitation experiment were fruit set and seed set. Fruit set is the proportion of flowers that set fruit. Seed set was estimated as the proportion of ovules that set seed in flowers that set fruit based on 4 ovules per flower. In external controls with two inflorescences, the response variable was the mean of the two. The data were analysed with a randomized-block ANOVA for each response variable.

Except where stated otherwise, measurements are reported as the mean ± 1 s.e.

RESULTS

Floral phenology and morphology

Flowering commenced in late April and peaked in late May 2007 (Fig. 2). On 26 May, 91.4 % of stems (n = 93) along transects were flowering, with a coincident peak in open flowers per inflorescence of 2.45 ± 0.15. *Isoglossa woodii* produced 215 ± 24 inflorescences per stem (n = 20) over the course of the flowering season, no new inflorescences being found after 16 June even in the minor resurgence of flowering in September to November which involved less than 10 % of stems.

Inflorescences comprised from 10 – 60 flower buds, the number developing into flowers being 25.8 ± 1.4 (n = 50). Flowering was acropetalous (flowering from the bottom of the infloresence upward). At a given moment an inflorescence could contain unopened flower buds, flowers in various stages of development, developing fruits, and mature pods.

The flowers of *I. woodii* are shortly tubular (mean 5.32 mm long, about half the length of the flower, Table 1). We found little variation in measurements among flowers (Table 1). Flowers produced an average of 10404 ± 550 pollen grains (n = 30), yielding a pollen:ovule ratio of 2601 ± 138. Pollen was available immediately upon anthesis and was quickly removed by insects. When flowers first open, the style is straight and the stigma not receptive, stigmatic activity commencing 2 – 4 d after the flower opened, once the stigma was recurved outwards (Fig. 1). This demonstrates that the species is protandrous, meaning that the male organs develop before the female organs.

Isoglossa woodii flowers offered a nectar reward at the base of the floral tube, though nectar was present in less than 6 % of flowers even after exclusion of insect visitors for 24 hours (Table 2). In flowers that did contain nectar, production over 24 h was 0.54 ± 0.08 µL (n = 50), the nectar having a sugar concentration of 34.2 ± 7.9 % (n = 5).

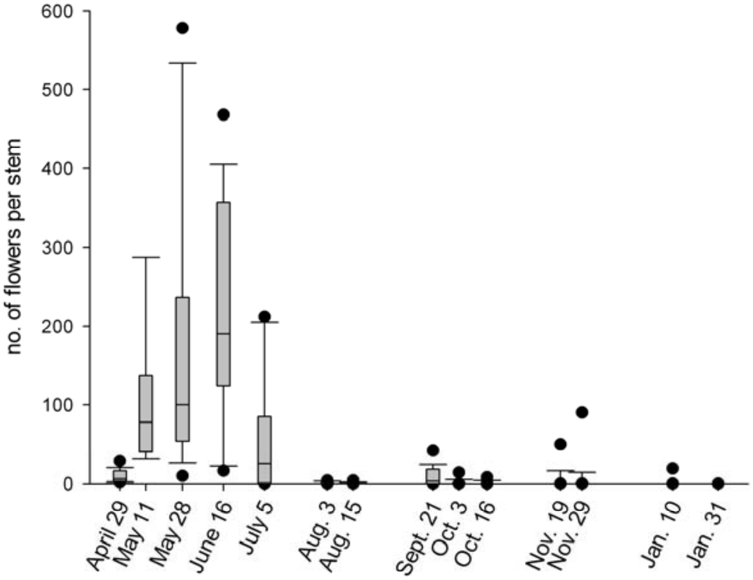

Fig. 2. Phenology of flowering in *Isoglossa woodii* based on 20 tagged stems. Data shown are: box - 25th, 50th and 75th percentiles; whiskers - 10th and 90th percentiles; dots - 5th and 95th percentiles.

Floral visitors

The African honey bee, *Apis mellifera adansonii* (Hymenoptera), was the only insect visitor to *I. woodii* flowers during the 80 10-min plot surveys. On average, foraging *A. m. adansonii* spent 2.6 s per flower and visited 1.51 ± 0.02 flowers per inflorescence (n = 1396; range 1 – 8). This was 73.1 ± 0.8 % of the open flowers on an inflorescence (n = 1319; range 12.5 – 150 %; 4 records [0.003 %] exceeded 100 % because individual flowers were visited more than one time). Recorded movements between inflorescences were 19.4 ± 0.9 cm (n = 1362; range 0 – 600). Other species of bees, butterflies, wasps and flies were noted visiting *I. woodii* flowers during incidental observations.

Apis m. adansonii collected at *I. woodii* flowers had high loads of pollen on all parts of the body and especially on the head and pollen sacs, almost all of which was *I. woodii* pollen (Table 3). The orientation of the stigma in *I. woodii* flowers ensures that pollen can be transferred from the head of *A. m. adansonii* to the stigmatic surface (Fig. 3).

Visit rates of *A. m. adansonii* to plots were assessed across temperatures ranging from 15 to 23 ºC and bees were active throughout this range (Fig. 4a). Temperature accounted for 11.5 % of the variance in bee visit rates, rates increasing with increasing temperature (P = 0.001). Inflorescence density accounted for only 0.04 % of the variance in visit rates (Fig. 4b; P = 0.86). The combined model (temperature + inflorescence density) performed only marginally better than temperature alone, accounting for 12.6 % of the variance.

Table 1. Measurements (in mm) of floral morphology in *Isoglossa woodii*. CV: coefficient of variation.

	mean	SD	n	CV
flower length	10.93	0.57	30	0.05
flower breadth	6.12	0.45	30	0.07
floral tube length	5.32	0.43	30	0.08
style length	7.05	0.46	30	0.07
stamen length				
upper	7.02	0.43	30	0.06
lower	6.02	0.43	30	0.07
anther breadth				
upper	1.97	0.35	30	0.17
lower	1.97	0.26	30	0.13
distance from anther to lower labium				
upper	2.98	0.33	30	0.11
lower	1.97	0.29	30	0.15

Table 2. Percentage of *Isoglossa woodii* flowers containing nectar (mean ± SE) at various times of the day (n = 30 stems per sampling period) and after 24-h nectar accumulation (n = 10 stems).

survey	% flowers with nectar
early morning	0.56 ± 0.56
midday	0.00 ± 0.00
late afternoon	4.22 ± 1.82
24 h accumulation	5.89 ± 1.25

Table 3. Mean pollen loads (± 1 SE) on *Apis mellifera adansonii* individuals collected from *Isoglossa woodii* stands (n = 10).

	number of *I. woodii* pollen grains	number of foreign pollen grains
head	800 ± 106	0 ± 0.0
upper body	49.7 ± 7.7	0 ± 0.0
lower body	196.8 ± 65.3	0 ± 0.0
pollen sacs	483 ± 143.8	0.2 ± 0.133

Fig. 3. The African honey bee *Apis mellifera adansonii* collecting nectar and pollen from an *Isoglossa woodii* flower. Note the position of the head relative to the anthers and stigma.

Breeding system

In the greenhouse pollination experiment, 37 % of cross-pollinated flowers and 39 % of self-pollinated flowers developed fruits, but selfed plants displayed markedly more variable responses to pollination than did cross-pollinated plants (Fig. 5). There was no fruit development in control flowers (Fig. 5). Differences among treatments were highly significant (Friedman's ANOVA $\chi^2 = 9.48$, n = 7, d.f. = 2, $P = 0.009$); *post-hoc* comparisons demonstrated significant differences only between the controls and each of the treatments ($P < 0.001$ in both cases). The self-compatibility index was 1.4 ± 0.7 (n = 82 flowers), samples with indices greater than 0.75 being described as self-compatible (Lloyd 1992). For both selfed and cross-pollinated flowers, growth of pollen tubes commenced between 24 and 48 h after pollination, with no obvious difference in speed of growth between selfed and cross-pollinated flowers.

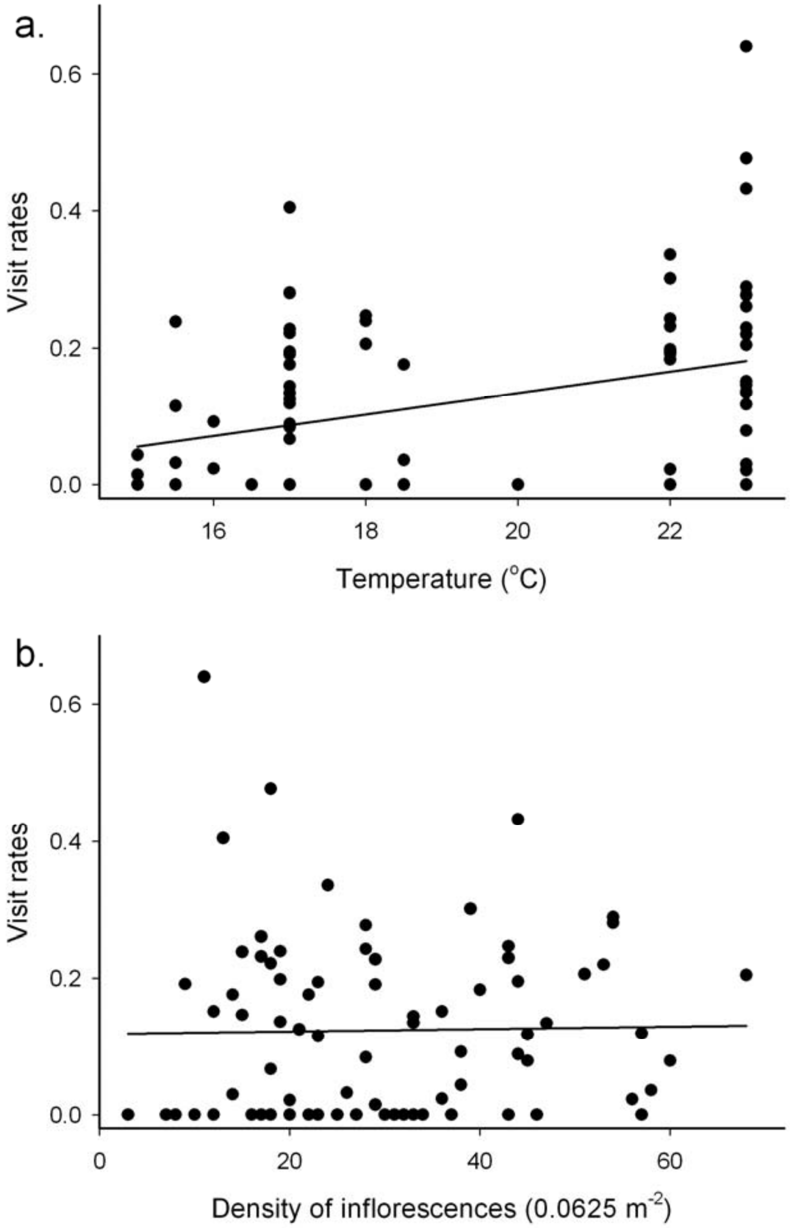

Fig. 4. Rates at which the bee *Apis mellifera adansonii* visited *Isoglossa woodii* flowers in 0.25 × 0.25-m plots compared to temperature (a) and the density of inflorescences (b). Data were collected between 09:40 and 14:00. The units of visit rates are $\log_{10}(x+1)$ (flowers / inflorescence / 10 min).

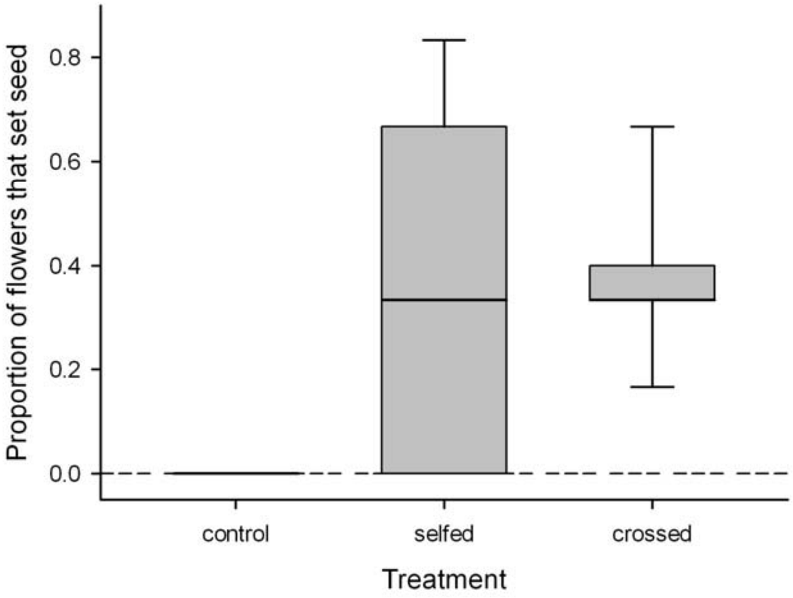

Fig. 5. Proportion of *Isoglossa woodii* flowers that set fruit after hand-pollination treatments. Data shown are median (line), 25th and 75th percentiles (box) and range (whiskers).

In the pollen limitation experiment, there was no significant difference between the pollen-supplemented inflorescences and the two controls for either fruit set ($F_{2,48}$ = 0.04, P = 0.96) or seed set ($F_{2,48}$ = 0.54, P = 0.59). Using treatments × blocks as replicates (n = 75), the proportion of flowers that set fruit was 0.60 ± 0.02 and the proportion of ovules that set seed was 0.48 ± 0.01. Notwithstanding that *I. woodii* has 4 ovules per flower, 89 % of fruits contained only 1 seed and none contained more than 2 seeds (mean of 0.95 ± 0.01, n = 710; 8.3 % with 0 seed, 2.8 % with 2 seeds).

DISCUSSION

In addition to being synchronised among years, flowering in *I. woodii* was synchronised among plants and among inflorescences within plants. However, flowers within inflorescences were markedly asynchronous, with typically less than 10 % of flowers (2.45 / 25.8) open at any time. This contrast is likely to reflect the tension between attracting pollinators and the risk of saturating them that underlies the evolution of floral display size in animal-pollinated plants (Harder & Barrett 1995; Benítez-Vieyra *et al.* 2006). Long-lived synchronous monocarps that are animal-pollinated, as in *I. woodii*, are a special case in that the tension is acted out with unusual strength at the scale of the group as well as at that of the individual.

Notwithstanding that *I. woodii* flowered over thousands of hectares, pollen supplementation in the field failed to increase the quite high proportion (60.5 %) of flowers that set fruit. Thus, *I. woodii* was successful in attracting pollinators without saturating them. It did so by attracting an abundant generalist pollinator, the African honey bee, to shallowly tubular flowers with a reward mainly of pollen. The rarity of pollen grains other than those of *I. woodii* on bees caught at *I. woodii* flowers suggests high fidelity by bees to the plant species. A synchronous monocarp and a masting tree that are animal-pollinated also attracted generalist insect pollinators (Pías & Guitián 2006; Sharma *et al.* 2008).

A mixed-mating strategy

Isoglossa woodii was self-compatible, having a mixed-mating strategy of the "single-flower type" (sensu Goodwillie *et al.* 2005). Selfing was geitonogamous (pollination of a flower with the pollen from another flower on the same plant). We detected no evidence of preferential outcrossing, though the dramatically different variability among plants in their response to pollination treatments suggests that selection pressure on self-compatibility is weak. Nevertheless, the species displayed a number of characters (acropetally, protandry, abundant production of pollen) that may promote outcrossing. The observed pollen-ovule ratio falls between the ranges for facultative and obligate outcrossing species reported by Cruden (1977) but may be elevated towards the range associated with obligate outcrossing by the provision of pollen as a reward for pollinators. As most flowers produced only one seed from four ovules, it is plausible that preferential outcrossing occurred after pollination (Teixeira *et al.* 2009), either by differential growth of pollen tubes or selective abortion of selfed ovules. This would greatly increase the chance that the seed will be the product of outcrossing and perhaps simultaneously increase fitness by limiting the division of resources among seeds. Selfed flowers may produce seeds of similar quantity but not quality as that of outcrossed flowers (Young 1982). Future studies on the relative performance of selfed versus outcrossed progeny, as well as the paternity of seeds resulting from mixed pollinations, would be required to confirm the hypotheses advanced.

Geitonogamous selfing may provide *I. woodii* with reproductive assurance in the face of the risks associated with synchronous monocarpy. However, support for the concept of selfing as a form of reproductive assurance for any plant remains equivocal because of the costs involved, and few models have successfully addressed its evolution (Goodwillie *et al.* 2005). Geitonogamy has been regarded as an accidental cost of the massed display needed to attract pollinators (Lloyd 1992; de Jong *et al.* 1993; Eckert 2000; Brunet & Sweet 2006b), and may also incur pollen- and seed-discounting costs (Goodwillie *et al.* 2005). However, reservations about the costs of geitonogamy are of reduced relevance to synchronously monocarpic species such as *I. woodii* for a number of reasons. First, synchrony among individuals facilitates attraction at the group level, providing a potentially beneficial mass-display without cost to the individual. We observed acropetally in *I. woodii*, which would minimise selfing. Second, one of the putative costs of geitonogamy, foregone future outcrossed reproduction (Goodwillie *et al.* 2005), is not incurred by monocarpic species. Third, investment in reproductive assurance is more likely in monocarpic than polycarpic species because of the enhanced benefits and not merely because of reduced costs. The effect may be further emphasized in long-lived synchronous monocarps by group-level selection (Wilson & Wilson 2007), especially where group members are relatives (Foster *et al.* 2006). Finally, the costs of pollen discounting may be minimised in *I. woodii* by the abundant production of pollen and its evident success in attracting pollinators, while the costs of seed discounting may be minimised by post-pollination processes that favour outcrossed seed.

Considerable transfer of pollen to flowers of the same individual is an almost inevitable and substantial consequence of synchronous monocarpy both because of the necessary size of individual floral displays and the requirement for a generalised and thus reliable pollination syndrome. Animal pollination may improve rates of outcross pollination compared to wind pollination – a potential compensation for the risks of pollinator satiation – but is limited by the inevitably generalist nature of the pollinators

(Brunet & Sweet 2006b). Our data on the movements of bees between *I. woodii* flowers demonstrates this consequence and conundrum in that many movements were between flowers within inflorescences or over distances that are mostly unlikely to preclude self pollination. A possible evolutionary response to this inevitability is to be self-compatible and make use of self-pollinations. Reproductive assurance is a simple and parsimonious explanation for selfing in synchronous monocarps, a possibility that may be enhanced by maximising rather than minimising rates of selfing, thus allowing plants to screen selfed ovules for deleterious alleles (Armbruster & Rogers 2004). An alternative is that selfed seeds may be a sacrificial component of satiation of seed predators (Ghazoul & Satake 2009). Given that the two primary hypotheses for the evolution of supra-annual flowering relate to pollination and satiation of seed predators respectively (Kelly 1994; Kelly & Sork 2002), the drivers of selfing in synchronous monocarps appear worthy of further investigation.

Monocarpy in the Acanthaceae

Monocarpy is particularly prominent among long-lived Acanthaceae, occurring in *Acanthopale laxiflora, Aechmanthera* spp., *Brillantaisia nitens* and *Mimulopsis solmsii* (Young & Augspurger 1991; Struhsaker 1997), in *Strobilanthes* spp. (Janzen 1976), in *Isoglossa* spp. (Van Steenis 1978), and possibly also in *Stenosiphonium* spp. (Carine & Scotland 2000). Many of these are synchronous monocarps. Sharma *et al.* (2008) reported a generalised bee pollination syndrome and high rates of fertilisation of flowers whether outcrossed or selfed in *Strobilanthes kunthianus* during a gregarious flowering event. Within *Isoglossa* as currently defined, *I. hypoestifolia* is iteroparous (C. Potgieter, pers. comm.) and, in stark contrast to *I. woodii*, has a specialised pollination syndrome involving a long-proboscis fly (Potgieter & Edwards 2005). The contrasts available within the family may provide an exceptional opportunity for evaluation of the evolutionary causes and consequences of synchronous monocarpy as a life history strategy in long-lived plants.

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CHAPTER 5

PREDATOR SATIATION AND RECRUITMENT IN A MAST FRUITING MONOCARPIC FOREST HERB

Format follows *Annals of Botany*

ABSTRACT

- *Background and Aims* Cross pollination and the satiation of seed predators have often been invoked to explain synchronous and mast reproduction in long-lived plants. In species with synchronous monocarpy, explanations for the death of parent plants has remained elusive. We investigate the roles of synchronous seeding and post-reproductive mortality of a perennial monocarpic herb (*Isoglossa woodii*) in Indian Ocean coastal dune forest in South Africa.
- *Methods* Predispersal seed predation was assessed by measuring fruit- and seed-set of inflorescences sprayed with insecticide, water and no spray treatments on 30 plants. Seed production per plant was measured on 30 plants. Seed predation was measured at different densities of *I. woodii* plants by monitoring removal rates of seed from the forest floor. Establishment of *I. woodii* seedlings was assessed in caged and uncaged 1 m × 1 m plots by monitoring growth and survivorship in understorey gaps and thickets.
- *Key Results* Fruit- and seed-set were similar between the spray treatments. An *I. woodii* stem produced 767.8 ± 160.8 seeds (mean ± SD) on dune crests and 1359.0 ± 234.4 seed on the foredunes (overall mean = 1083.4 ± 144.4) during a reproductive event. With stem densities ranging from 8 m^{-2} on foredunes to 10.4 m^{-2} on dune crests, seed rain produced as many as 6142 seed m^{-2} on dune crests and on foredunes, 14134 seed m^{-2}. Seed predation rates ranged from 36 to 50% on dune crests and dune slacks, respectively, but was not related to seed abundance or stem density. Seedling recruitment was greater in forest patches with synchronously dying adult plants (understorey thickets) than in

natural gaps (no *I. woodii*) in the understorey. However, the growth of seedlings transplanted to a site where *I. woodii* was mid-way through its life-cycle was greater in gaps than in thickets, although survivorship was similar.

Conclusions The selective advantage of masting in *I. woodii* derives from its ability to satiate predators. Post-seeding mortality of adult plants facilitates seedling establishment. Satiation of seed predators and benefits of seedling establishment may have driven the evolution of synchronous monocarpy in *I. woodii*.

Key words: synchronous flowering**,** understorey gaps, *Isoglossa woodii*, seed predation, seedling establishment

INTRODUCTION

The best known evolutionary advantages of synchronous reproduction or masting in plants are pollination efficiency for wind-pollinated species and satiation of seed predators (Silvertown, 1980; Ims, 1990; Kelly, 1994; Kelly and Sork, 2002; Schauber *et al*., 2002). In many perennial plants exhibiting synchronous reproduction, reproduction is supra-annual, with intervening periods ranging from three to over one hundred years (Janzen 1976, Keeley & Bond 1999, Sharma *et al*., 2008). Masting supra-annually gains an evolutionary advantage if longer reproductive cycles result in higher fitness compared to annually reproducing plants. In masting monocarpic species of Acanthaceae, satiation of seed predators and facilitation of seedling establishment by the synchronous death of parent plants have been proposed as the ultimate causes of synchronous reproduction (Janzen, 1976; Struhsaker, 1997). Here we investigate the effect of the density and age of adult plants on seed predation and seedling establishment of a monocarpic herb (*Isoglossa woodii*, Acanthaceae) in subtropical forests of eastern South Africa.

Isoglossa woodii is a native semi-woody herb that dominates the understorey of coastal dune forests (Griffiths *et al*., 2007; Tsvuura *et al*., 2007). The species exhibits synchronous reproduction and mortality events on a 4 to 7 year cycle (Griffiths *et al*., 2009), which are common features in many species of the Acanthaceae (Tweedie, 1965; 1976; Janzen, 1976; Struhsaker, 1997; Sharma *et al*., 2008).

The outcrossing hypothesis (Janzen 1976; Augspurger 1980; Stephenson 1982) suggests that a large visual display associated with synchronous flowering increases the chances of cross-pollination and benefits the parent plant by producing high quality seed. However, this benefit of synchronous flowering in animal-pollinated species may be negated by pollinator satiation on an individual plant so that there is less movement between plants (Kelly 1994, Kelly & Sork 2002), which would be ultimately maladaptive. In the case of self-compatible species synchronous flowering may also lead to geitonogamous self-pollination, which provides reproductive assurance but conflicts with the benefits of outcrossing. Consequently, masting is often associated with wind-pollination because it increases the chances of successful pollination as well as enhancing outcrossing (Smith *et al.,* 1990; Schauber *et al.,* 2002).

The predator satiation hypothesis (Janzen, 1976; Silvertown, 1980; Augspurger, 1981) proposes that synchronous reproduction in long-lived species produces more seed than can be consumed by seed predators in mast years, and starves the seed predators in non-seeding years. Where predation pressure is high, a fitness advantage is predicted for individual plants that flower synchronously with the rest of the population. If predation rates are high then asynchronous lineages will eventually be eliminated from the population. By removing asynchronously flowering individuals from the tails of the seeding distribution, predation pressure, through stabilising selection, effectively selects for synchronous reproduction.

A third hypothesis is that interspecific competition particularly regarding the timing of establishment of seedlings may have driven the evolution of synchrony in monocarpic species (Struhsaker, 1997). The dominance, in terms of cover, of monocarpic species is maintained by synchronised mass reproduction and seedling establishment, which excludes seedlings of other species beneath the parent plants. As the parent plants die their seedlings are released from competition. This argument suggests that individuals that release their seedlings from competition in this way gain a fitness advantage leading to synchronised seedling establishment and ultimately synchronised reproduction, which in turn outcompetes other species thereby maintaining herb dominance.

The evolution of seedling-facilitative, post-reproductive mortality of adult plants requires that seeds, and eventually seedlings, of an individual plant end up directly below the parent (Janzen, 1976), which is feasible and possibly adaptive for passively dispersed seed (Foster, 1977; Gadgil and Prasad, 1984).

In this study we experimentally examined two hypotheses of the evolution of synchronous flowering in *I. woodii*, and explored mechanisms that determine its distribution and cover in the forest. First, we determined whether insect consumers feeding on flowers and developing seed and fruit may limit seed production by subjecting developing inflorescences to insecticide-spray and no-spray treatments. Second, we measured seed production and tested the predator satiation hypothesis at a local scale by monitoring removal of *I. woodii* seed from seed stations located in areas of different *I. woodii* densities. The prediction is that seed predation will be greater at sites where the number of seed in the soil seed bank is lower. Third, we tested the hypothesis that mortality of parent plants facilitates seedling establishment (‘seedling facilitation hypothesis,’ *sensu* Foster, 1977; Struhsaker, 1997) using a seedling transplant experiment situated at two locations where the *I. woodii* population was in vegetative or post-reproductive phases. This latter experiment was designed to test which factors can prevent establishment of *Isoglossa*, to show which selection pressures are likely to be important for explaining synchronous flowering. We make the following predictions on the seedling-facilitation hypothesis: (1) seedling establishment in the vegetative phase-population will be greater in areas with no *I. woodii* cover than in areas with an *I. woodii* cover, and (2) seedling establishment in the post-reproductive phase-population will be greater in areas with *I. woodii* cover than in areas with no *I. woodii* cover.

MATERIALS AND METHODS

Study species and populations

Isoglossa woodii (Acanthaceae) is a widespread herbaceous to semi-woody broad-leafed understorey plant in subtropical coastal dune forests of eastern South Africa and south-eastern Mozambique. Isolated patches of *I. woodii* can be found up to 10 km or more from the coast, varying in extent from a few stems covering a few square metres to thickets extending over several hundred hectares. In some areas the species dominates the understorey of coastal dune forests, covering at least 65 % of the forest floor (Griffiths *et* *al.*, 2007). Following flowering and seed dispersal, *I. woodii* dies back and the population regenerates from seed. Several populations of *I. woodii* occur in the iSimangaliso Wetland Park in KwaZulu-Natal Province, South Africa. Two of these, at Cape Vidal (28˚16' S, 32˚29' E) and Mapelane Nature Reserve (28˚24'32"S, 32˚25'17"E), cover hundreds of hectares of the understorey in the coastal dune forest. The Cape Vidal population flowered from March-October 2007, and seedling establishment after flowering was measured at this site. The Mapelane population last had a mass flowering event in 2003, was in mid-cycle at the time of this study, and in this population we measured seedling establishment under established *I. woodii* stands.

Seed set, pre-dispersal flower and seed predation

Seed production may be limited by consumers feeding on the parent plant, insufficient pollen or numbers of pollinators, limited resources for plant growth and reproduction, and environmental conditions (Louda and Potvin, 1995). Without compensatory flowering or seeding (McNaughton, 1986), the lifetime fitness of individual plants may be reduced by predators feeding on the inflorescence, seed or seedlings (Louda and Potvin, 1995). To determine whether predator satiation is an advantage for the lifetime fitness of *I. woodii* we estimated seed production with and without predation.

We measured pre-dispersal flower and seed predation using a modification of the design by Louda and Potvin (1995). Thirty *I. woodii* plants at least 5 m apart were tagged on the foredune, dune crest, backdune and in dune slacks. Density, height and basal diameters of the plants were measured, and on six inflorescences on each plant three treatments were applied: insecticide spray (Chlorpirifos, Agro-Serve (Pty) Ltd, Silverton, South Africa); water spray (as a control for the physical effects of spraying); and no spray. Treatments were initiated when inflorescences were still developing before any flower buds were evident. Spraying was carried out once per fortnight. After the main flowering event and before the dispersal of seed, all inflorescences were harvested and each kept separately in paper envelopes. In the laboratory, we counted the numbers of flowers and fruits developed on each inflorescence. From each inflorescence we dissected eight fruits and counted the number of seed in each. Fruit set (number of fruits/number of flowers) was calculated for each inflorescence as well as seed set (number of seed/number of ovules) for each fruit.

Seed production

Seed production was measured before dispersal when fruits were maturing. Thirty *I. woodii* plants of different sizes were randomly selected for measurement, ten each from the foredune, dune crest and dune slack. For each plant we measured its size (number of stems, stem diameter and height) and counted the number of inflorescences. Ten inflorescences representing the range of sizes present on the plant were harvested from each plant. We counted the number of flowers and fruits developed on each inflorescence. For five inflorescences from each plant we dissected five fruits and counted the number of seed in each. We calculated the mean fruit- and seed- set for each plant. The number of seed produced by each plant was estimated as the product of the number of inflorescences and the mean fruit set and mean seed set per inflorescence.

Seed predation

Post-dispersal seed predation was investigated using a modification of a method by Wurm (1998) of placing seeds in Petri dishes at sites and monitoring their removal rate. Seeds were collected during the *I. woodii* flowering event and the experiment was conducted just as *I. woodii* seed fall was coming to an end in July–August 2007. We compared seed removal rates in areas with different stem and inflorescence densities, including *I. woodii* gaps. Since the number of seed already on the ground may affect seed removal rates, we estimated the number of seed in the soil by sieving soil from four samples per site. Each site was a 1-m^2 quadrat where we counted stems, measured their heights and numbers of inflorescences, and estimated browsing. Then we measured seed densities in the top 1cm of soil in four Petri dish (circle with diameter = 9 cm) samples covering 0.25 m^2. In the centre of the quadrat we placed two Petri dishes 20 cm apart, filled with seed-free sand. We randomly selected one of the two Petri dishes for placing seed for measurement of removal rates. The other dish was retained as a control with no seed to allow estimation of seed fall into the treatment dish. Eight seeds were placed on the soil surface of the dish. Sites were marked, and the dishes were checked for removal or addition of seed three times at 10 to 13 day intervals between July and August 2007. Removed seeds were replaced while surplus seeds were removed.

There were 28 to 46 seed stations per sampling period. Stations were located at two sites on foredunes, two on dune crests and two on dune slacks. Each site had four to 21 stations.

Establishment of I. woodii *seedlings in gaps during die-back*

At Cape Vidal, the understorey consists of large areas of *I. woodii* cover and some patches without *I. woodii* cover (Griffiths *et al*., 2007). We investigated why *I. woodii* gaps occur on the forest floor at Cape Vidal. In each of 15 sites paired into gap and non-gap (thicket) plots, we transplanted 16 *I. woodii* seedlings in a grid layout into each of two 1 m × 1 m sub-plots between 09 September and 29 November 2007. We established two more 1-m^2 sub-plots in the thicket nearby but within 10 m of the edge of the gap. One sub-plot in each of the two plots per site was covered by a cage to prevent large mammalian herbivores from disturbing the seedlings. Ten days after transplantation, seedlings that had died were assumed to have died of transplant shock and were therefore replaced. We recorded seedling survival at three-month intervals and also recorded evidence of browsing (apex of shoot cut off) and severe herbivory ($\geq$ 50% or more of leaf tissue eaten). Final measurements were carried out in early November 2008.

Establishment of I. woodii *seedlings beneath adult conspecific plants*

In addition to determining whether recruitment failure is responsible for the absence of *I. woodii* in some patches of the forest, we examined whether *I. woodii* seedlings were capable of establishing beneath mature plants, to test the hypothesis that synchronous reproduction and the associated synchronous mortality of adult plants facilitates recruitment. In late March 2008 we transplanted *I. woodii* seedlings from Cape Vidal to 10 sites 40 km south at Mapelane Nature Reserve. Each site consisted of three 1 m × 1 m sub-plots whose centres formed the corners of a 5 m-sided equilateral triangle. One sub-plot was always located in a natural gap in the *I. woodii* cover, another in the centre of an artificial 2 m × 2 m gap created by removing the *I. woodii* to ground level, and a third located under stands of mature *I. woodii.* The number of stems removed in the artificial gap and the size of the natural gap was recorded. In each sub-plot, 16 *I. woodii* seedlings with heights 80–120 mm (but choosing similarly-sized seedlings in any one sub-plot) were planted in a grid layout. Seedlings that died of transplant shock were replaced after ten days. *I. woodii* stems re-growing into artificial gaps were removed regularly. Eight months after transplanting we measured survivorship and height growth of seedlings.

Data analysis

Fruit- and seed-set data were analysed using analysis of covariance (ANCOVA) with topographic location, *I. woodii* stem density, height, and basal diameter as covariates. Relationships between the predictor variable and any significant covariate were explored using regression.

For seed production data, we calculated Pearson correlation coefficients to explore the nature of relationships between plant size and each of seed-set, fruit-set and number of inflorescences per plant. Significant correlations were further explored using linear regression.

Seed predation data were arcsine square-root transformed to fit a normal distribution of residuals before analysis (Kéry and Hatfield, 2003). We used ANCOVA to determine whether there were effects of topography, site, *I. woodii* stem density and height, number of inflorescences per *I. woodii* stem, soil seed bank density, and mean date of sampling as predictor variables. Topographic location was defined as a fixed factor, with site as a random factor nested within it. All other predictor variables were categorised as covariates. To determine whether there may be spatial density effects on predation, we also calculated neighbourhood-scale densities by averaging the size of the soil seed bank, stem density and number of inflorescences for a station and its two nearest stations and included these additional covariates into the ANCOVA model.

In both transplant experiments, seedling growth was calculated as (final height-initial height)/initial height, while survivorship was the proportion of the 16 seedlings that survived in each sub-plot. Growth data were square-root transformed while survivorship data were arcsine square-root transformed to restore normality of residuals.

At Cape Vidal, seedling growth and survivorship were analysed among the 15 sites using a split plot design, with site as a block with two plots: gap and thicket. Each of these was split into a caged and uncaged sub-plot. At Mapelane, growth and survivorship of seedlings beneath adult conspecific plants were compared among three *I. woodii* habitats made up of thickets, artificial clearings and natural gaps of *I woodii* cover using analysis of covariance with gap size, stem density and stem height of cleared *I. woodii* plants as covariates. We explored the nature of the relationship between any significant covariate with the response variable using simple linear regression. All analysis were carried out in SPSS v. 15 (SPSS 2007).

RESULTS

Seed set, pre-dispersal flower and seed predation

Fruit set was similarly high between treatments ($F_{2,\ 161} = 0.005$, $P = 0.99$, Table 1) but varied between topographic locations ($F_{1,\ 161} = 6.492$, $P = 0.01$). There was some evidence for greater fruit-set on the foredune and backdune than in the dune slack while the other covariates were not significant (data not shown). Seed set was also similar between the spray treatments ($F_{2,\ 161} = 1.440$, $P = 0.24$, Table 1) and none of the covariates was significant ($P > 0.05$ in all cases). Of the 1247 fruit examined, 74.3 % contained only 1 seed, 4.5 % had 2 seeds and the rest had no seed.

Table 1. Mean (±se, %) fruit and seed set of inflorescences of *I. woodii* treated with insecticide spray, water spray and no-spray treatments

Treatment	Fruit set	N	Seed set
Insecticide	61.1 ± 2.4	434	39.2 ± 1.6
Water spray	61.4 ± 2.3	427	42.3 ± 1.6
No spray	61.1 ± 3.1	386	46.7 ± 1.4

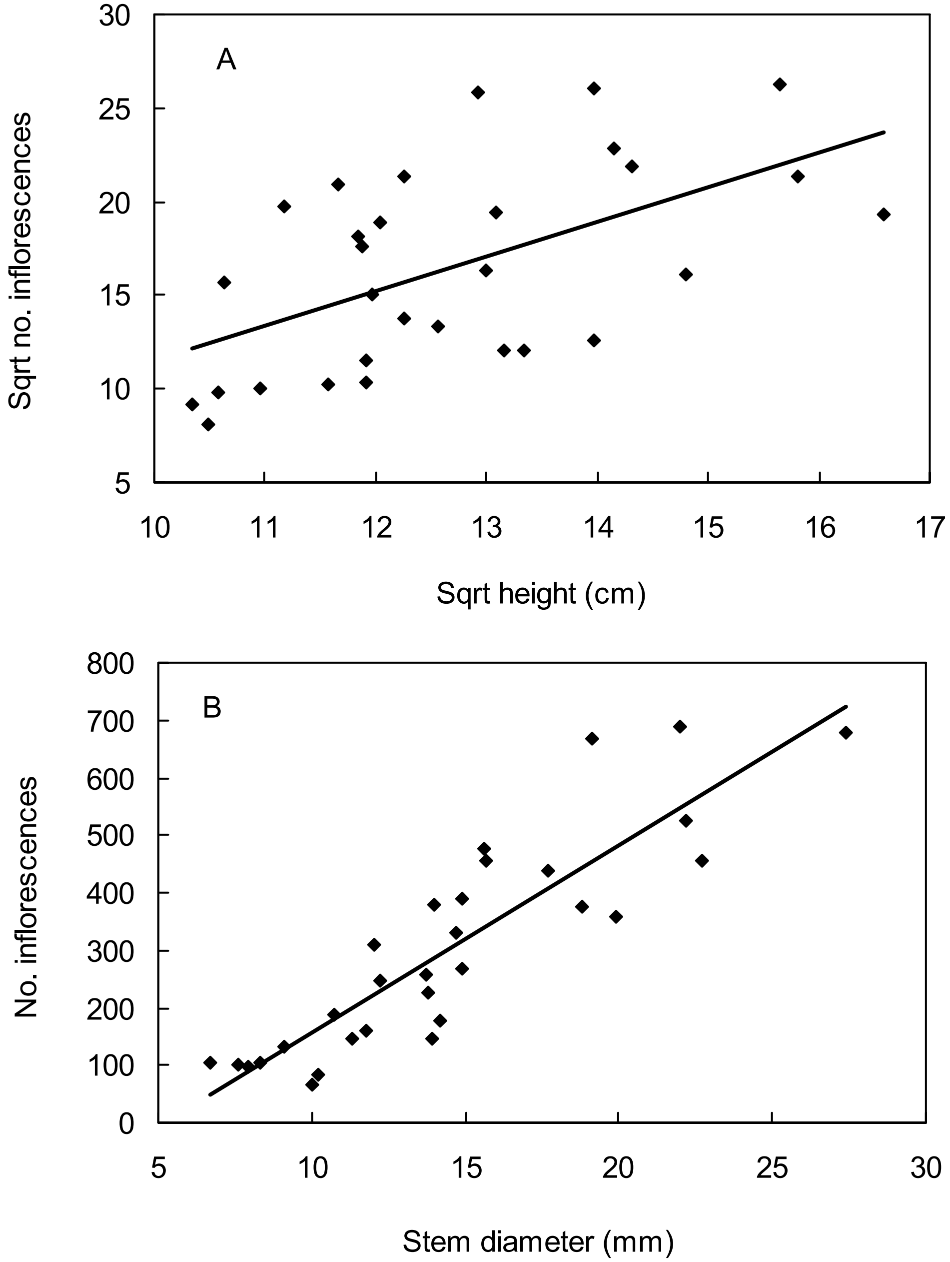

Fig. 1. Number of inflorescences on different sizes of *I. woodii* plants. In (A), the relationship is y = 1.84x – 6.87 ($F_{1,\,28}$ = 14.72, P = 0.001, r^2 = 0.32) and in (B), y = 32.49x – 167.9 ($F_{1,\,28}$ = 99.18, P < 0.0005, r^2 = 0.78)

Seed production

Both fruit set and seed set were not correlated with plant height and stem diameter ($-0.035 < r < 0.235$, $P > 0.05$, N = 30 in all cases). However, the number of inflorescences was positively correlated with both plant height and stem diameter (Fig. 1).

An *I. woodii* plant produced 767.8 ± 160.8 seed on dune crests and 1359.0 ± 234.4 seed on the foredunes (overall mean across topographic locations = 1083.4 ± 144.4, n = 30). Considering that stem densities range from 8 stems m^{-2} on foredunes to 10.4 stems m^{-2} on dune crests, a square metre of ground surface on dune crests can receive 6142 seed and on foredunes, 14134 seed. At other topographic locations, seed production was within the range for dune crests and foredunes.

Seed predation

Losses of seed to predation ranged from 36 % on dune crests to 50 % in dune slacks but was similar across these topographical locations ($F_{2, 6} = 3.402$, $P = 0.10$) and across sites ($F_{5, 30} = 1.48$, $P = 0.22$, Table 2). There were no effects of the size of the soil seed bank, stem density or plant height ($P > 0.05$ in all cases, Table 2). The main predator of *I. woodii* was an ant species, *Pheidole* spp. (Formicidae), with occasional sightings of *Meranoplus* spp. (Formicidae) at seed stations.

Establishment of I. woodii *seedlings in gaps during die-back*

After 11 months, seedling growth was greater in the *I. woodii* thicket than in the gaps ($F_{1, 14} = 7.18$, $P = 0.018$) and in the caged plots than in the open ($F_{1, 668} = 13.42$ $P < 0.001$, Table 3a). These trends were evident only eight months after the experiment was initiated (Fig. 2). Caging and the thicket habitat had higher seedling survivorship than uncaged and gap habitats but the difference was greater between caged and uncaged plots (Fig. 3). These patterns show a greater effect of herbivory on seedling survivorship than gaps *per se*.

Table 2. Analysis of covariance for predation of *Isoglossa woodii* seed at Cape Vidal. Seed stations were located at six sites on three topographic locations. Site is a random factor, nested in the fixed factor topography. All other variables are covariates. Density 3, seed bank 3 and number of inflorescences 3 were derived by averaging their values at each station and the nearest two stations

Source	df	MS	*F*	*P*
Topography	2	0.204	3.40	0.10
Residual	6	0.060		
Site(topography)	5	0.063	1.48	0.22
Seed bank	1	0.036	0.84	0.37
Height	1	0.060	1.43	0.24
Mean sampling date	1	0.149	3.52	0.07
Density	1	0.002	0.04	0.84
Number of inflorescences	1	0.001	0.03	0.88
Seed bank 3	1	0.030	0.72	0.40
Density 3	1	0.034	0.81	0.38
Number of inflorescences 3	1	0.073	1.73	0.20
Residual	30	0.042		

Data were arcsine square-root transformed before analysis

Table 3. Growth (a) and survivorship (b) of *I. woodii* seedlings grown in caged and uncaged sub-plots in *I.w*+ and *I.w*- habitats at Cape Vidal

(a)

Source of Variation	df	MS	*F*	*P*
Site	14	0.474	2.06	0.09
Residual	14	0.230		
Habitat	1	1.566	7.18	0.02
Residual	14	0.218		
Site*Habitat	14	0.230	7.27	<0.001
Cage	1	0.424	13.42	<0.001
Habitat*Cage	1	0.011	0.36	0.55
Residual	668	0.032		
Overall Residual	699	0.049		

(b)

Source of Variation	df	MS	*F*	*P*
Site	14	0.050	0.69	0.75
Residual	14	0.072		
Habitat	1	0.292	4.05	0.06
Residual	14	0.072		
Site * Habitat	14	0.072	4.25	0.001
Cage	1	0.227	13.35	0.001
Habitat * Cage	1	0.011	0.65	0.43
Residual	28	0.017		
Overall Residual	59	0.046		

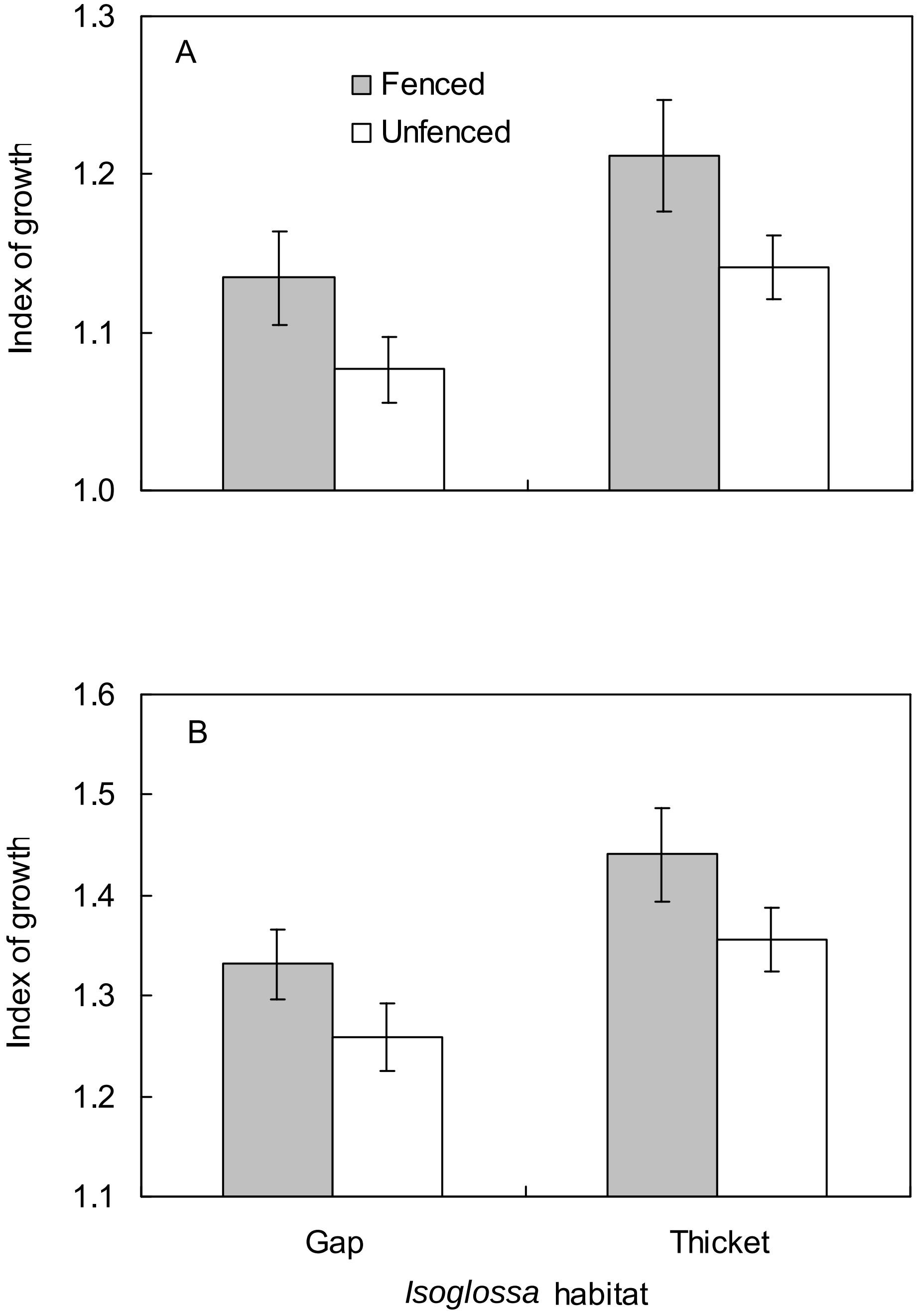

Fig. 2. Mean (± 1se) growth of *I. woodii* seedlings transplanted into fenced and unfenced plots in *I. woodii* gaps and thickets at Cape Vidal after (A) eight months and (B) 11 months. Index of growth is based on square-root transformed values.

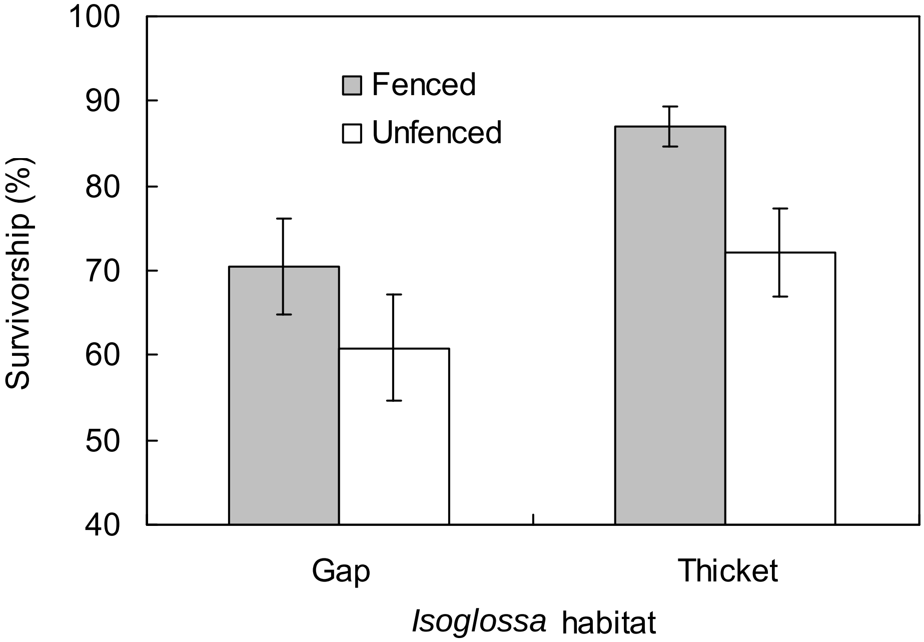

Fig. 3. Mean (± 1se) survivorship of seedlings in herbivore-protected (fenced) and herbivore-unprotected (unfenced) plots under *Isoglossa woodii* present (thicket) and *I. woodii* absent (gap) habitats at Cape Vidal.

Establishment of I. woodii *seedlings beneath adult conspecific plants*

Eight months after the experiment was initiated at Mapelane, seedling growth was more suppressed in the thicket than in the gaps ($F_{2, 432} = 25.92$, $P < 0.001$, Table 4), and was highest in the artificial gaps (artificial gap > natural gap > thicket, Fig. 4). Of the three covariates, only gap size was important ($F_{1, 432} = 9.27$, $P = 0.002$). In natural gaps, seedling growth was not related to gap size ($r = 0.071$, $P = 0.135$, N = 438). There was a trend for greater survivorship in the natural gaps than in the thicket and artificial gaps (Fig. 4). The number of *I. woodii* stems cleared in the artificial gaps was a significant covariate (Table 4) but this was also not correlated with survivorship ($r = 0.034$, $P = 0.859$, N = 30).

Table 4. Growth (a) and survivorship (b) of *I. woodii* seedlings grown in natural and artificial gaps of *I. woodii*, and thicket, at Mapelane.

(a)

Source of Variation	df	MS	*F*	*P*
Habitat	2	0.959	25.92	<0.001
Stem density	1	0.009	0.23	0.63
Stem height	1	0.001	0.02	0.90
Gap size	1	0.343	9.27	0.002
Residual	432	0.037		
Total	438			

(b)

Source of Variation	df	MS	*F*	*P*
Habitat	2	0.103	2.97	0.07
Stem density	1	0.180	5.18	0.03
Stem height	1	0.076	2.19	0.15
Gap size	1	0.015	0.44	0.52
Residual	24	0.035		
Total	30			

DISCUSSION

Seed predation

We found little evidence for predispersal flower and seed predation in a synchronously reproducing *I. woodii* population. Inflorescences that received supplemental pollen and those that received no additional pollen did not differ in fruit set i.e., not pollen limited (Griffiths *et al*., 2009), and values of fruit set from this study (61 %) did not differ from the 60 % of Griffiths *et al*. (2009). As reproduction in the species is not pollen limited, fruit set did not differ between treatments and was similar to the natural circumstance. Similarly, at 39-47 % seed-set is consistent with the fact that most fruit are one-seeded, and that a flower has 2 to 4 ovules (Balkwill and Wellman, 2000; Griffiths *et al.*, 2009).

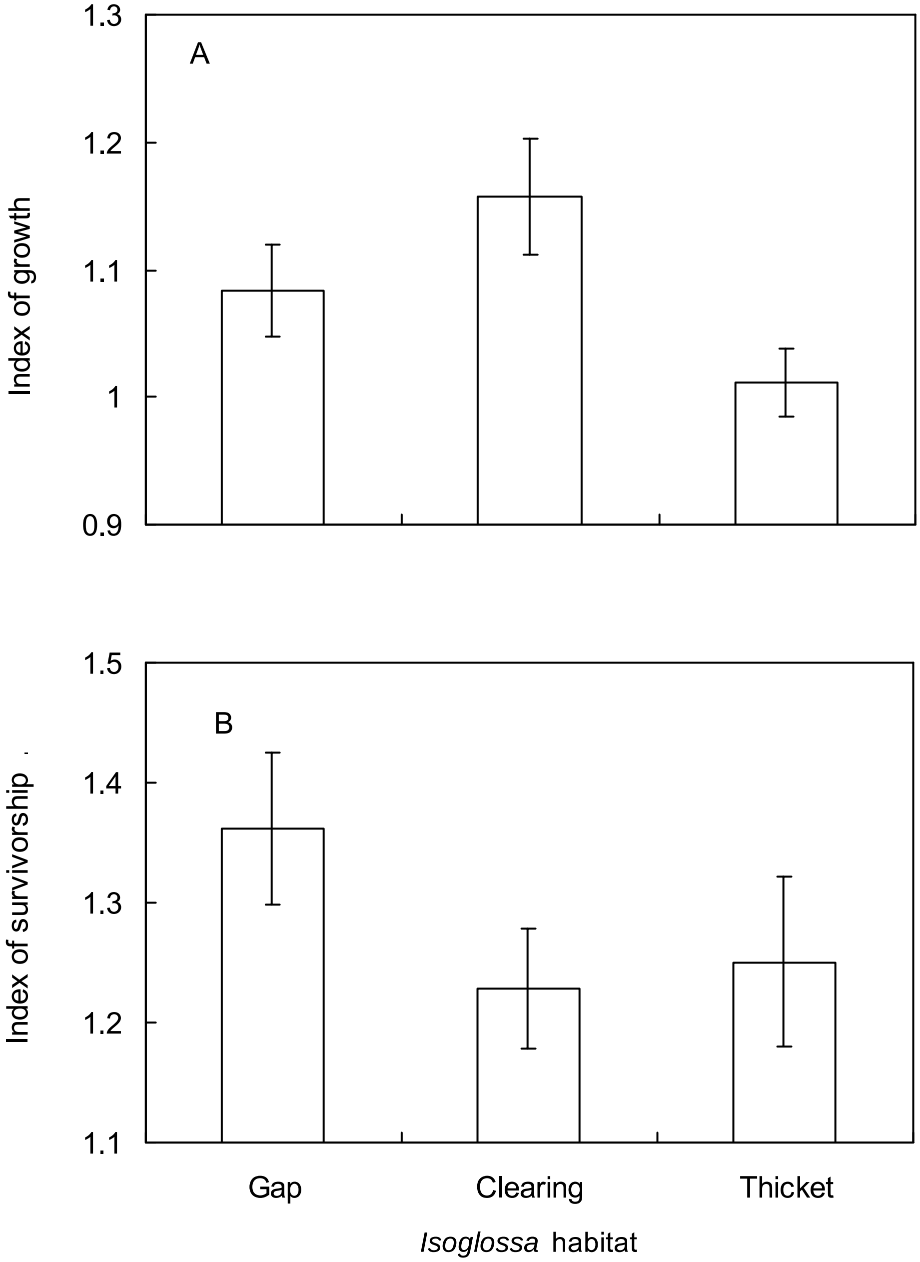

Fig. 4. Mean (± 1se) growth (A) and survivorship (B) of *Isoglossa woodii* seedlings from Cape Vidal grown at three *I. woodii* habitats at Mapelane.

Only an occasional (<3%) insect-damaged seed was observed and predispersal seed predation was low. Attempts to obtain seed from isolated plants flowering out of synchrony with the rest of the population in 2005 at St. Lucia, ca. 35 km south of Cape Vidal, and in 2006 at Cape Vidal, were largely unsuccessful because of predispersal seed predation (Z. Tsvuura, pers. obs.). The latter strengthens the case for mast reproduction events satiating seed predators. At St Lucia, post-seed dispersal plants were visited several times until they senesced but no seedlings germinated beneath the dying canopies. However, during masting of the Cape Vidal and St Lucia populations in 2007 and 2008 respectively, seed could readily be obtained and a carpet of seedlings emerged within two months of seed fall. The low levels of predispersal seed predation may therefore be caused by masting, which satiates seed predators.

Silvertown (1980) identified three interdependent elements to the predator satiation hypothesis. The first is that there should be enough seed produced for predators to consume and spare some. Second, that the interval between masting events should be sufficiently long to result in marked declines of the predator population (or at least prevent sustaining a large predator population), and finally that reproduction should be synchronised within the population and between sympatric species sharing the same seed predators. In our study system the first condition is realised: seed fall ranged from 6100 to 14100 m^{-2}, depending on plant size, topographic location and/or stem density. The period between masting events for the *I. woodii* population at Cape Vidal was 7 years, which is sufficiently long to prevent the maintenance of large populations of invertebrate seed predators that specialise on *I. woodii* seed. The main post-dispersal predator of *I. woodii* seed, *Pheidole* spp., is a generalist seed-eating ant species (Hölldobler and Wilson, 1990; Andersen, 1991), and as such is unlikely to manifest a numerical response to the stages of the reproductive cycle of *I. woodii*.

Reproductive synchrony was evident in May 2007 when more than 90 % of the Cape Vidal *I. woodii* population flowered synchronously (Griffiths *et al*., 2009). We observed no other species of the Acanthaceae in flower at the same time as *I. woodii*. A similar finding of non-synchronised flowering of sympatric monocarps was reported in India and at Kibale Forest in Uganda but was associated with predator satiation in India but not at Kibale (Janzen, 1976; Struhsaker, 1997). Synchronised mast fruiting among many species of the Dipterocarpaceae has also been explained in terms of predator satiation (Janzen, 1974; Ashton *et al*., 1988).

Post-dispersal predation of *I. woodii* seed was neither related to the abundance of seed on the ground nor to the density of plants. In fact, we found similar levels of predation from site to site, demonstrating no fine-scale (seed bank size and plant density) or coarser-scale (site) predation effects. At both scales of measurement of predation, seed availability surpassed seed losses, which is consistent with expectation according to the predator satiation hypothesis and provides additional evidence for it. O'Dowd and Gill (1984) compared seed predation between a site where fire induced mass release of seed from *Eucalyptus delegatensis* canopies and an unburnt site, and found greater seed losses to ants at the unburnt site. Moreover the ratio of ants to seeds was greater at the unburnt than burnt site, which lent further support to the notion that predators were satiated at the burnt site (O'Dowd and Gill, 1984). Silvertown (1980) reported an increase in predispersal seed survival with an increase in seed crop size for 24 of 59 data sets and concluded that these data sets supported the predator satiation hypothesis. Our value (> 97 %) of predispersal seed survival during a synchronous reproductive event may be associated with predator satiation (see above).

Post-reproductive senescence and seedling establishment

The establishment of *I. woodii* seedlings by the death of adult *I. woodii* plants may occur through increased light levels (Read *et al*., 2006), through improved aeration and penetrability of the soil as a result of dying and decomposing roots, or through improved water penetration and availability (see Vitousek and Denslow, 1986). Seedlings recruitment was greater under dying plants than in gaps, which suggests that the monocarpic strategy facilitates recruitment of seedlings. Gap sites may be unsuitable for recruitment of *I. woodii* in general (*sensu* Griffiths *et al*., 2007). However, at 6.3 ± 0.5 μmol m^{-2} s^{-1} the light compensation point of the species is lower than background levels of light in understorey gaps (Z. Tsvuura, unpublished data), which should not preclude *I. woodii* establishment. Nevertheless, our finding of greater *I. woodii* establishment beneath dying conspecific plants concurs with Foster (1977), who suggested that post-reproductive death of *Tachigalia versicolor* trees at Barro Colorado Island in Panama facilitates establishment of its seedlings. In fact, Kitajima and Augspurger (1989) found enhanced growth and survivorship of *T. versicolor* seedlings growing beneath dying conspecific adult trees than under the canopies of living conspecific and non-conspecific trees. Light gaps created by the death of a monocarp plant may be open for colonisation by seedlings of pioneer species, but for a species exhibiting prolific reproduction, the vacant sites are rapidly taken over by emerging seedlings of the species. The absence of seed dormancy in these monocarps (Young, 1982; Taylor and Inouye, 1985) increases the probability that regeneration sites will be occupied by conspecific seedlings.

Struhsaker (1997) suggested that death of parent plants of monocarpic Acanthaceae allows seedlings of the species to competitively swamp seedlings of other species, thereby maintaining the dominance of the monocarp. The death of mature *I. woodii* plants may also facilitate seedling establishment through competitive release. However, facilitative effects of understorey plant mortality may be accompanied by suppressive effects. For example, Paul *et al*. (2004) have shown that collapsing dead stems of a dominant undercanopy shrub, *Acanthus pubescens,* smothers and suppresses tree seedlings in logging gaps. This is in sharp contrast to the death and eventual collapse of an *I. woodii* plant: after seed dispersal, an *I. woodii* plant starts to die, beginning with the inflorescences and inflorescence stalks, followed by the small thin branches that support inflorescences, then proceeds gradually to the larger branches and main stem. By the time the larger stem dies, the inflorescences and inflorescence-bearing stalks have fallen off so that at any one time very little biomass falls off an *I. woodii* plant. This mortality pattern is unlikely to smother *I. woodii* seedlings but could in fact enhance their chances of establishment as suggested for the mortality pattern of the monocarpic tree *Tachigalia versicolor* (Foster, 1977).

We found greater establishment of *I. woodii* seedlings in the artificial and natural gaps than beneath adult plants at Mapelane. This lends further support to the notion that the mortality of post-reproductive plants enhances establishment of their seedlings. Patterns of survivorship showed that the natural gaps were associated with higher seedling survivorship than the artificial gaps and *I. woodii* thicket, which had similar survivorship. This suggests mortality due to below-ground competition, perhaps for water as most mortality occurred in the dry season after winter rainfall.

As *I. woodii* reproduces synchronously within and among plants and produces large numbers of seed at supra-annual scales, the selective advantage of masting in the species probably derives from its ability to satiate predators. Seedling establishment in the species is facilitated by synchronous mortality of post-seeding plants. We conclude that satiation of seed predators and the benefits of seedling establishment may have driven the evolution of synchronous monocarpy in *I. woodii*.

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CHAPTER 6

COMPETITIVE EFFECTS OF A DOMINANT UNDERSTOREY HERB *ISOGLOSSA WOODII* (ACANTHACEAE) ON TREE SEEDLINGS IN A SUBTROPICAL DUNE FOREST IN SOUTH AFRICA

This chapter has been written following the format of the *Journal of Ecology*.

Summary

1. Suppression of seedlings of canopy trees by the understorey has been proposed as an important ecological filter with implications for tree diversity and dynamics.
2. In greenhouse and field competition experiments, we used four canopy species of coastal dune forest (*Diospyros natalensis, Euclea racemosa, Sideroxylon inerme* and *Apodytes dimidiata*) to examine the relative competitive effects of the pervasive and monocarpic understorey herb *Isoglossa woodii* on seedling performance of these species under contrasting light and nutrient conditions.
3. In the greenhouse, total seedling biomass decreased with density of *I. woodii* and light levels but not with site fertility. The magnitude of biomass suppression with competitor density and light levels was similar among tree species. Consequently there was no discernable hierarchy of competitive ranking of the tree species.
4. The relative growth rate (RGR) of seedlings decreased at higher densities of *I. woodii* and increased at higher nutrient levels but was not affected by variation in light conditions. Above-ground biomass decreased at higher densities of *I. woodii* and at higher light levels but increased at higher nutrient levels. In the field, there were no density effects on RGR and biomass, but above-ground biomass increased with density of *I. woodii*.
5. *Synthesis*. Size asymmetric competition for light may be the major driving force in tree seedling-*I. woodii* interactions in coastal forest. Species show no hierarchy of

competitive ability but may exhibit equivalent responses to competition from an understorey dominant, leading to species coexistence and maintenance of species diversity.

Key-words: above-ground biomass, monocarpy, pairwise species interactions, competitive response, competitive effect, relative interaction index, light gradient, soil fertility

Introduction

A central outcome of competitive interactions among plant species is the reduced performance of subordinate species (Keddy *et al.* 1997, 2000). Such interactions in the understorey may determine the trajectory of regeneration by overstorey tree species. For example, in temperate and tropical forests, the understorey may act as a selective ecological filter that determines community characteristics such as species composition, productivity and species interactions (George & Bazzaz 1999, 2003; Gonzalez *et al.* 2002; Benitez-Malvido 2006).

The mechanisms of suppression of tree seedlings by understorey vegetation can be related to above-ground exploitative competition resulting in light limitation (Horsley 1993; Clinton & Vose 1996; George & Bazzaz 2003; LaFrankie & Saw 2005; Griffiths *et al.* 2007) and to non-competitive processes such as providing habitat for seed predators (Wada 1993; George & Bazzaz 1999, 2003), deep litter impeding seedling germination or establishment (George & Bazzaz 1999, 2003; Farris-Lopez *et al.* 2004) and transfer of insects and pathogens to seedlings (Denslow *et al.* 1991). Suppressive effects of understorey plants on woody seedlings may also operate through below-ground mechanisms such as competition for soil moisture and nutrients (Clinton & Vose 1996; Nilsen *et al.* 2001) and through the production of allelopathic compounds (Clinton & Vose 1996; Nilsen *et al.* 1999). In this study we explore the synergistic effects of above- and below-ground competition between seedlings of canopy trees and an herbaceous plant that dominates the forest understorey.

Competitive interactions in plant communities involving mature understorey plants and seedlings of canopy trees are likely to be size-dependent with a disproportionately high uptake of resources by the larger plants. This phenomenon has been termed size asymmetric competition by Schwinning & Weiner (1998). However, Callaway & Walker (1997) suggest that positive interactions in which understorey plants facilitate performance of seedlings co-occur with negative interactions by which seedlings are suppressed. Here we are concerned with asymmetric competition for light and nutrient resources in the superficial soil horizons, where roots of woody seedlings and adventitious roots of understorey plants are in close proximity.

The severity of asymmetric competition among plant species may be related to environmental factors such as habitat productivity. In fact, the central assertion of the tripartite model of primary plant strategies is that the importance of competition increases under conditions of high productivity and neighbour density, and declines in low productivity habitats (Grime 1988, 2001). Others argue that competition remains the primary determinant of vegetation structure and assert that declining levels of productivity changes the nature of competition from above-ground to below-ground (Tilman 1988; Wilson & Tilman 1993; Tilman 1997, 2007). In this study we use soil fertility to represent habitat productivity as a determinant of plant biomass. We also consider how habitat productivity may influence root biomass, rather than only to include its influence on above-ground biomass.

The understorey of subtropical coastal dune forests in South Africa is dominated by the native semi-woody herb *Isoglossa woodii* C.B. Clarke (Acanthaceae). The species is locally associated with lower tree seedling density and species richness, and reduces light levels from 8 % to < 1 % beneath its cover (Griffiths *et al.* 2007). The effect of *I. woodii* on woody seedlings is probably multifold, with possible above-ground and below-ground suppressive effects on tree seedling abundance and diversity. The high growth rate of the species suggests that above-ground size-asymmetric competition results in overtopping and shading of relatively slow-growing tree seedlings. Below-ground access to resources by tree seedlings may be pre-empted by *I. woodii*'s extensive rooting system (MJ Lawes unpublished). In addition, the consistency of any competitive hierarchy driven by *I. woodii* is complicated by the species' monocarpic life history, which may cause its dominance over seedlings of other species during its vegetative phase but subordinate status to other species during its reproductive and early growth phases.

We hypothesise that *I. woodii* competes with tree seedlings for light and nutrients, thereby reducing their performance (growth and survival) and subsequently influencing dune forest tree dynamics. From this hypothesis we make the following predictions: (1) at high levels of nutrients, competition between woody seedlings and *I. woodii* will be greater than at low levels of nutrients; (2) at low light levels, *I. woodii*'s fast growth rate preempts the light resource and outcompetes woody seedlings resulting in a greater competitive effect at low light; and (3) the suppressive effect of *I. woodii* on woody seedlings increases with density of *I. woodii*.

Materials and methods

STUDY SPECIES

Isoglossa woodii is a broad-leaved herbaceous to shrubby understorey dominant in coastal dune forests of the iSimangaliso Wetland Park (SWP) in KwaZulu-Natal, South Africa. The species covers 65 - 95 % of the understorey (Griffiths *et al*. 2007). With a monocarpic life history, *I. woodii* grows vegetatively for several years to a height of ± 2 m, after which it reproduces synchronously, dies and regenerates from seed within a year of seeding. Its dominance in the understorey may have a pervasive effect on recruitment of tree seedlings (Griffiths *et al*. 2007; Tsvuura *et al*. 2007).

Four tree species that are representative of the forest tree community were selected to investigate the effect of the herb on community dynamics of coastal forest. *Diospyros natalensis* (Harv.) Brenan is a monopodial, multi-branched tree that dominates the canopy tree stratum (Nzunda *et al*. 2007) at Cape Vidal in the SWP. *D. natalensis* seedlings at the cotyledon stage are abundant in the forest. *Euclea racemosa* Murray is a multi-stemmed sub-canopy tree that attains a height of 12 m. While the species is fairly abundant in the forest (4.7 % of tree basal area), seedlings are not common, except in gaps in the *I. woodii* cover that occur beneath adult *E. racemosa* trees. *Sideroxylon inerme* L. is also multi-stemmed, grows to a height of 17 m and is a canopy dominant (8.53 % of tree stem basal area) with prolific resprouting potential. *S. inerme* seedlings occur at low density in the forest (Nzunda *et al*. 2007). *Apodytes dimidiata* E.Mey. ex Arn. is a less common (1.25 % of tree stem basal area) single-stemmed canopy tree that grows to 13 m. Seedlings of this species are uncommon in the forest.

EXPERIMENTAL DESIGN

Greenhouse competition experiment

To determine the competitive effects of *I. woodii* on tree seedlings, we grew seedlings of *D. natalensis, E. racemosa, S. inerme* and *A. dimidiata* with *I. woodii* cuttings derived from shoots of *I. woodii* plants. All plants were grown in sterilised sand collected from the study site, in 4 L pots with perforated bases and under two light levels (see below) in the greenhouse (mean daytime temperature of 24 °C from May-August, and 27 °C from October-April).

We used a three-factor crossed and randomised experimental design with nutrients, light and *I. woodii* density as main factors. A 50 % Hoagland's nutrient solution (Hewitt 1966) containing low (1 %) and high (10 %) nitrogen concentration was used as a nutrient source and applied every third day. Levels of nitrogen were based on soil samples taken in the field. Plants were provided with additional water to keep the substrate moist, and were also watered to soil saturation with tap water once a week to reduce accretion of salts from the nutrient solution.

The two light levels applied were *shade* (1.6 ± 0.5 % photosynthetically active radiation [PAR] at 1200 h on a sunny day) and *sun* (13.5 ± 1.1 % PAR). Commercially available 80 % shadecloth was used to simulate shade conditions. The shade cloth was supported on metal frames (170 cm × 128 cm × 121.5 cm: length × width × height, respectively). Each treatment was replicated four times so that each shade structure represented a replicate. There were 12 pots per shade structure of the *shade* plants (4 tree species × 3 densities of *I. woodii*, see below) and 12 pots of s*un* plants outside each shade structure but on the same bench.

To determine the competitive effects of *I. woodii* on tree seedlings, it is necessary to measure how well each species grows on its own and in combination with the other species. The competition experiment therefore followed an additive design (Goldberg & Scheiner 2001), whereby the density of seedlings was kept constant while that of *I.*

woodii was varied. With an additive design, the magnitude of competitive effects can be reliably determined (Goldberg & Fleetwood 1987; Austin *et al*. 1988). Three density treatment levels were used: one seedling and no *I. woodii* cuttings; one seedling with one *I. woodii* cutting; and one seedling with three *I. woodii* cuttings. The three densities of tree seedlings represent three possible competition intensities: no competition (seedling only, no *I. woodii*); low competition (one seedling, one *I. woodii* cutting); and high competition (one seedling, three *I. woodii* cuttings). The zero competition density (one seedling only) also allows for separation of shade effects without the confounding effects of below-ground interactions between *I. woodii* and tree seedlings. There were four replicates per treatment group, resulting in 192 samples (4 seedling species × 2 nutrient levels × 2 light levels × 3 competition intensities × 4 replicates).

Seedlings of *A. dimidiata* and *S. inerme* were derived from seed germinated in the greenhouse, and were 9 and 12 mo old, respectively, when they were transplanted. *E. racemosa* and *D. natalensis* seedlings were collected from the forest at Cape Vidal, where a few trees of these two species were monitored from fruit fall to seedling emergence. *E. racemosa* and *D. natalensis* seedlings were approximately 2 and 12 mo old, respectively, at time of collection. At time of transplanting, the height of the shoot and the stem diameter of each seedling were measured. Seedlings had grown to stem heights of 64.9 ± 1.5 mm (*E. racemosa*), 73.1 ± 1.5 mm (*D. natalensis*), 105.8 ± 2.5 mm (*S. inerme*), and 128.7 ± 3.2 mm (*A. dimidiata*) (mean ± 1SE, based on 48 seedlings in each case).

Cuttings of *I. woodii* were taken from shoots of actively growing adult plants collected at Cape Vidal. Shoots were rooted in sand in a mist-house until they were transplanted at 46 days old. Average height of the cuttings was 98.5 ± 1.3 mm ($n = 272$).

We checked that seedlings of each species allocated to treatments were of similar size at the start of the experiment using ANCOVA. There were no initial differences among seedlings of each species in treatment groups ($p > 0.05$ in all cases, data not shown).

To reduce position effects on the plants, pots were shifted within the shade or sun structures at 3 mo intervals during the course of the experiment. After 16 mo, we measured the height and basal diameter of seedlings and *I. woodii* plants. Plants were harvested, separating above-ground from below-ground components and roots were carefully rinsed clean. Leaves, stems and roots of seedlings and *I. woodii* were oven dried at 70 ºC for 48 h.

Field competition experiment

We replicated the greenhouse competition experiment in the forest at Cape Vidal. Seedlings of *E. racemosa* and *D. natalensis*, and cuttings of *I. woodii* from greenhouse stock were used. Plots of 1 m × 1 m and a 2 m wide shade free buffer zone around the perimeter of each plot were established on the forest floor by cutting back *I. woodii* stems to a 3 cm height. In each plot, *I. woodii* plants were uprooted, and the plot was subdivided into four subplots by trenches (30 cm deep × 10 cm wide). All roots were severed; to reduce lateral root growth into neighbouring subplots, each subplot was lined with 30 cm wide plastic sheeting and the trench refilled. In each plot, we randomly allocated seedlings and *I. woodii* cuttings or their treatment groups into the subplots, representing the three competition intensities described above (see *Greenhouse competition experiment*). We used 12 replicates per treatment group in 96 subplots (4 treatment groups × 12 plots × 2 seedling species). To prevent large mammal herbivory, the 1 m^2 plots were covered with 50 cm high cages made from wire mesh (50 mm × 50 mm). To further reduce lateral root in-growth, plots were trenched at bimonthly intervals, when any emerging seedlings and weeds were also removed. For each seedling in the field experiment, their size at establishment was measured. Seedlings were not different in either measure of size ($p > 0.05$ in all cases, data not shown). After 11 mo, the height and basal diameter of seedlings and *I. woodii* were measured. Plants were harvested and oven dried as for greenhouse plants.

DATA ANALYSIS

Greenhouse competition experiment

We compared the performance of tree seedlings growing with *I. woodii* under the light and nutrient treatments using ANCOVA with total dry weight (hereafter referred to as total biomass), height-based relative growth rate (RGR) and shoot mass fraction (SMF: shoot weight/total seedling weight) as the dependent variables. We used biomass because it has been shown to be a good predictor of competitive ability (Keddy *et al.* 2000), while RGR is indicative of height growth. SMF was used for two reasons: to indicate whether competitive effects may be reflected in allocation patterns to above- and below-ground biomass, and secondly to check on RGR, which is based on height increment of the shoot but does not include horizontal growth. Some species invest substantially in horizontal growth to enhance light capture (King 1990, 1996). For total biomass we calculated a relative interaction index (RII) for each species using a modification of Armas *et al.* (2004) as follows:

$\text{RII (biomass)} = (\text{Biomass}_{\text{treatment}} - \text{Biomass}_{\text{monoculture}})/$
$(\text{Biomass}_{\text{treatment}} + \text{Biomass}_{\text{monoculture}})$,

where $\text{biomass}_{\text{treatment}}$ refers to the biomass of seedlings grown with *I. woodii* neighbours and $\text{biomass}_{\text{monoculture}}$ is the biomass of seedlings grown without *I. woodii* neighbours. For each species in each treatment group, a mean performance at monoculture was calculated and used to derive an RII for each seedling grown under potential competition at the same level of light and nutrients. This was repeated for all treatment groups. The RII is an index whose values range from -1 to +1, with negative values indicating competition with surrounding vegetation and positive values indicating facilitation by the surrounding vegetation (Armas *et al.* 2004). Biomass-based RIIs, relative growth rate (expressed as mm of growth per meter of stem per day) and shoot mass fraction were analysed using Model III ANCOVA, with light and nutrients as the main factors, seedling species as a random factor and density of *I. woodii* as a covariate. As the biomass of seedlings grown at monoculture was used in intermediate calculations of RII, biomass data consisted of two levels of the covariate compared to three levels with RGR and SMF data. A significant effect of density would indicate competition between seedlings and *I. woodii*. The natural-logarithm transformation was used to normalise or homogenise residuals of the data where necessary. We also examined whether the relationships between density and each response variable (i.e., their interaction) differed between groups as a test for the assumption of homogeneity among slopes (Engqvist 2005). When the interaction between a factor and the covariate was not significant, the effect of competition from *I. woodii* was assumed to be the same among treatment groups (parallel linear regressions between different treatments) and we performed a second analysis excluding the covariate interaction term which increases the degrees of freedom due to the error term and increases the power of the test. All statistical analyses were carried out using SPSS v.15 (SPSS 2007).

Field competition experiment

For seedlings grown in the field, the density of *I. woodii* was the only treatment imposed. RGR, SMF and RII derived from seedlings' total biomass were used to compare seedling performance. Data were analysed using Model II ANCOVA with species as a random factor and density of *I. woodii* as a covariate.

Results

Greenhouse competition experiment

A higher density of *I. woodii* resulted in greater suppression of tree seedling biomass (Fig. 1). The nutrient treatment had no discernable effect on seedling biomass while the light treatment was marginally important (Table 1). Seedling RGR increased with higher nutrients but declined with greater density of *I. woodii* at both levels of light (Table 2, Fig. 2). In the absence of *I. woodii*, seedling RGR was greater at high than at low light levels but when grown with *I. woodii*, RGR was marginally depressed at high rather than at low light levels (Fig. 2). Seedlings grown with *I. woodii* allocated less biomass to shoots compared to control seedlings grown without *I. woodii* (Fig. 3). High light levels also reduced seedling SMF, while high nutrient levels increased SMF (Table 2, Fig. 3).

Considering the effect of *I. woodii* on each species separately, biomass reduction was marginally greater (more negative) at higher density for *D. natalensis* and *S. inerme* (Fig. 4). The magnitude of suppression of seedling RGR varied among species (Table 2). *A. dimidiata* had a high growth rate, and when grown with *I. woodii*, its growth rate was comparable to that of singly grown *D. natalensis* and *S. inerme* (Fig. 5a). Allocation of biomass to shoots decreased with density of *I. woodii* for all tree species except *A. dimidiata* but was similar at both densities with *I. woodii* present (Fig. 5b).

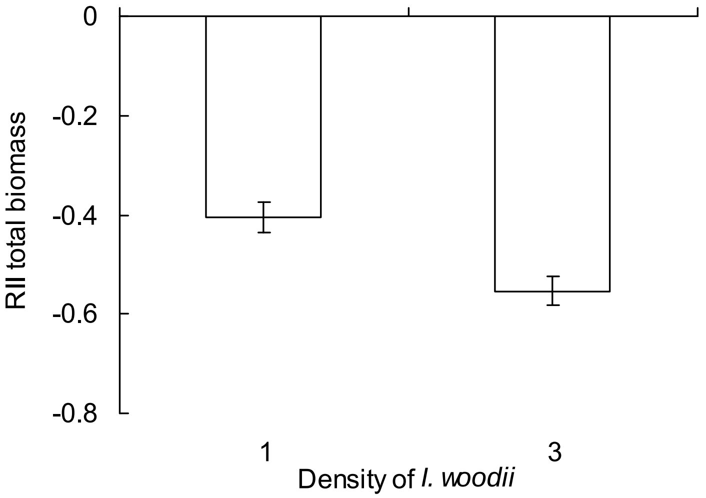

Fig. 1. Relative interaction index (RII), in terms of total biomass, of tree seedlings grown at two densities of *I. woodii* in the greenhouse.

Table 1. Analysis of covariance (ANOVA) for the effect of light, nutrients, species and the covariate density of *I. woodii* on total biomass-based relative interaction index of tree seedlings.

Source of Variation	df	MS	*F*	*P*
Light (L)	1	2.60	6.29	0.087
Residual	3	0.41		
Nutrients (N)	1	0.03	0.59	0.500
Residual	3	0.05		
Species (S)	3	0.12	0.30	0.825
Residual	3	0.40		
Density	1	0.70	39.96	<0.001
Residual	111	0.02		
L × N	1	0.07	1.03	0.385
L × S	3	0.41	6.20	0.084
N × S	3	0.05	0.79	0.573
Residual	3	0.07		
L × N × S	3	0.07	3.79	0.012
Residual	111	0.02		
Overall residual	111			

Table 2. Analysis of covariance (ANCOVA) for the effect of light, nutrients and the covariate density of *I. woodii* on relative growth rate (mm m^{-1} day^{-1}) and shoot mass fraction of tree seedlings. Light and nutrients are the main effects, with species as a random factor and density of *I. woodii* as a covariate

Dependent Variable	Source of variation	df	MS	*F*	*P*
Relative growth rate	Light	1	1.81	1.510	0.307
	Residual	3	1.20		
	Nutrients	1	5.14	30.615	0.012
	Residual	3	0.17		
	Species	3	11.74	9.241	0.040
	Residual	3	1.27		
	Density	1	28.75	149.319	<0.001
	Residual	167	0.19		
	L × N	1	0.23	20.807	0.020
	Residual	3	0.01		
	L × S	3	1.20	11.044	<0.001
	Residual	162	0.11		
	L × D	1	3.23	16.767	<0.001
	Residual	167	0.19		
	N × S	3	0.17	15.267	0.025
	Residual	3	0.01		
	S × D	3	1.83	9.525	<0.001
	L × N ×S	3	0.01	0.057	0.982
	L × S ×D	3	0.50	2.594	0.054
	Residual	167	0.19		
	Overall residual	167			
Root mass fraction	Light	1	0.89	15.644	0.029
	Residual	3	0.06		
	Nutrients	1	0.03	61.959	0.004
	Residual	3	0.001		
	Species	3	0.05	1.675	0.336
	Residual	3	0.03		
	Density	1	0.16	41.127	<0.001
	L × N	1	0.001	0.297	0.589
	L × S	3	0.06	14.379	<0.001
	N × S	3	0.001	0.129	0.943
	S × D	3	0.04	8.878	<0.001
	Residual	174	0.004		
	Overall residual	174			

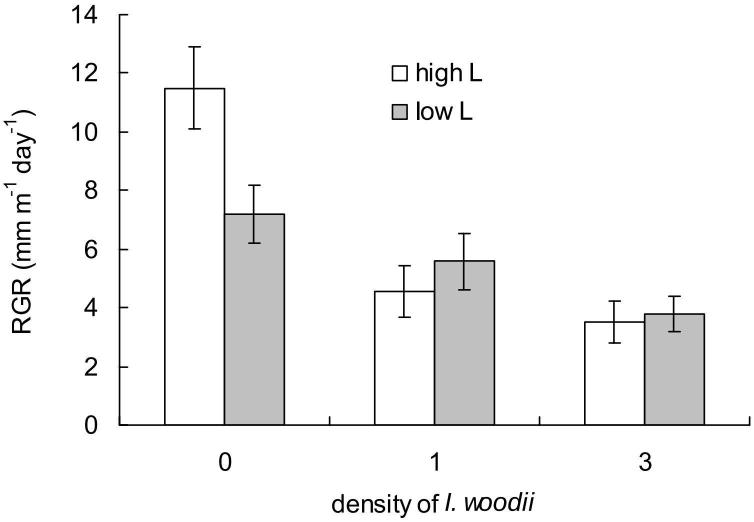

Fig. 2. Relative growth rate (mean±1SE, mm growth. m^{-1}.d^{-1}) of seedlings grown under two light (L) levels at three densities of *I. woodii* in the greenhouse.

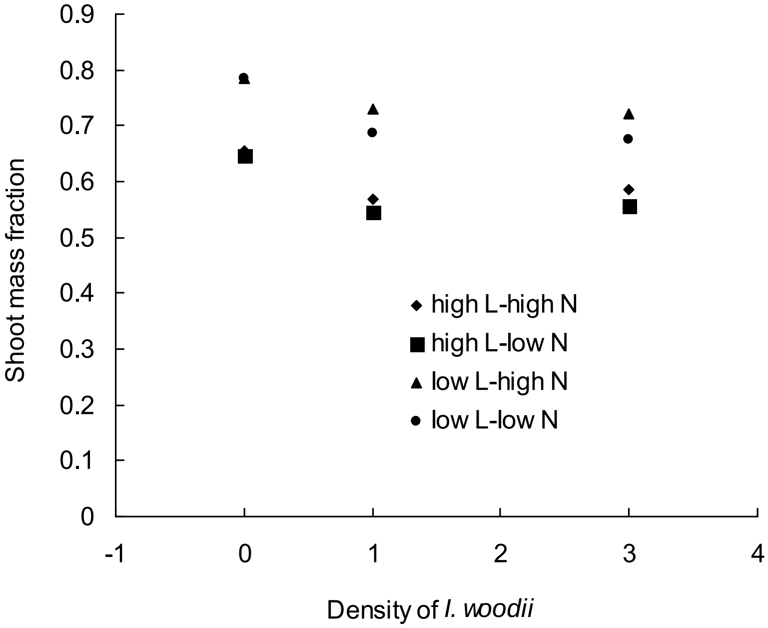

Fig. 3. Shoot mass fraction of tree seedlings grown at three densities of *I. woodii* under two combinations of light (L) and nutrients (N) in a greenhouse.

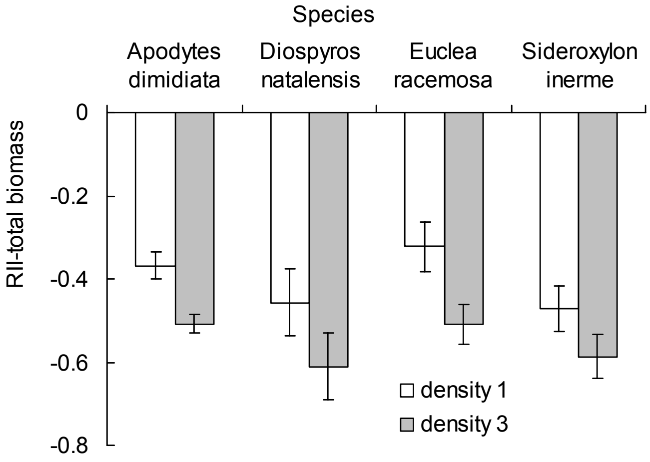

Fig. 4. Relative interaction index (mean ± 1SE) in terms of biomass for seedlings grown at two densities of *I. woodii* in the greenhouse.

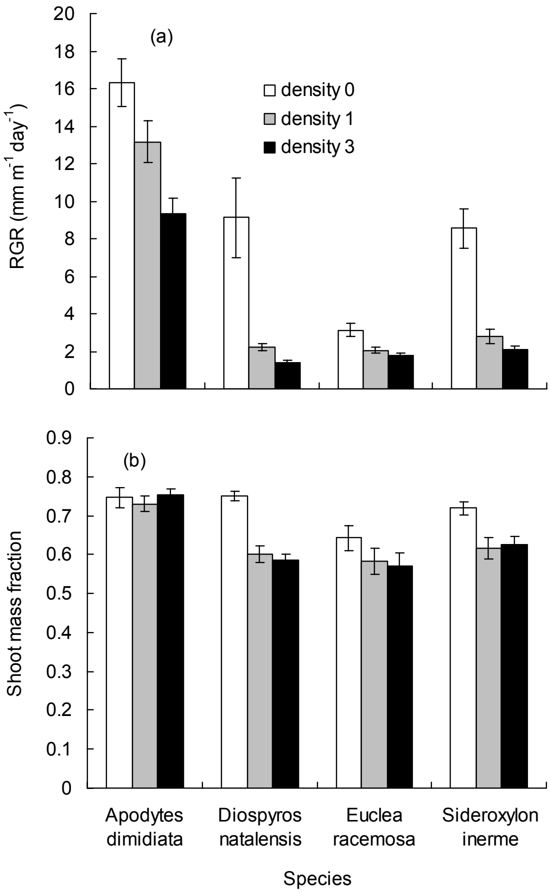

Fig. 5. Mean (±1SE) relative growth rate ((a), mm m^{-1} day^{-1}) and shoot mass fraction (b) of tree seedlings grown singly (density 0) or with *I. woodii* (densities 1 and 3).

Field competition experiment

Unlike in the greenhouse experiment, *I. woodii* had no significant effect on either total biomass or RGR of tree seedlings in the field ($P > 0.05$ in both cases, Table 3). Allocation of biomass to shoots was, however, greater at higher than lower density of *I. woodii* (Fig. 6), and was also different between the two species (Table 3).

Discussion

We found significant effects of *I. woodii* density in determining the total biomass, relative growth rate and shoot mass fraction of tree seedlings, with higher densities of *I. woodii* resulting in greater suppression of seedlings. There were marginal effects of light in influencing biomass of seedlings grown with *I. woodii*. These findings suggest that *I. woodii* competes with and reduces tree seedling performance, which supports our hypothesis of competition between tree seedlings and the herb. Contrary to our prediction of increased competition at high nutrient levels, we found similar competitive responses of seedlings at the two nutrient levels, which concurs with some studies (e.g. DiTommaso & Aarssen 1991, Wilson & Tilman 1993) and not with others (e.g. Campbell & Grime 1992, Keddy *et al*. 1997, 2000). In fact, Goldberg (1994) and Grace (1995) refute Campbell and Grime's findings and assert that the use of competition indices shows that competition intensity was not affected by soil fertility, which concurs with our findings. Although the soil at Cape Vidal is relatively infertile with a mean organic carbon content of 3 % (Z. Tsvuura, unpublished data), competition between *I. woodii* and tree seedlings was similar between the nutrient levels, suggesting that competition is not affected by site productivity, but is affected by herb density and light conditions reaching the seedlings.

The decreased allocation to shoot biomass with increased *I. woodii* density is commensurate with an increased biomass allocation to roots. A higher allocation to root biomass may indicate larger investment in carbohydrate storage pools, which are important for buffering against stress (Kobe 1997). Seedlings of sprouting tree species in the same coastal dune forests have higher allocation to root biomass and root total non-structural carbohydrate stores than non-sprouting species (Nzunda 2008). However, here we used both sprouting and non-sprouting species whose biomass allocation patterns were similar. Increased allocation to root biomass may therefore be a general phenomenon of competition among small numbers of plants in pots. Contrary to this finding, the field experiment showed evidence for increasing above-ground biomass of seedlings with increased density of *I. woodii*, which may indicate greater above-ground competition in the wild. Greater biomass allocation above-ground may be achieved by minimizing allocation to root and carbohydrate stores (Bond & Midgley 2003; Knox & Clarke 2005; Schwilk & Ackerly 2005), and suggests that root competition is lower in conditions of low light. A plant growing in high light conditions should invest more resources in acquiring resources that are not as abundant, a suggestion that is consistent with our finding of greater below-ground biomass at higher light levels.

The importance of light in determining seedling biomass and shoot mass fraction suggests that light may be a mechanism of competitive suppression of tree seedlings by *I. woodii*. Plants of *I. woodii* in 15 % full sun branched copiously and rooted from nodes that came into contact with the soil substrate. Competition with seedlings may therefore have been through space pre-emption or for other below-ground resources such as soil moisture. The mechanisms of competitive suppression from *I. woodii* may vary among species. For example, George & Bazzaz (2003) identified three mechanisms of understorey suppression of four tree species by ferns in North American hardwood forests. The mechanisms varied between species and ranged from low levels of soil exposure through competition for light to higher seed predation under fern cover (George & Bazzaz 1999, 2003).

In a plant community, competitive effects co-occur with and may be reduced by facilitative effects (Callaway 1995; Callaway & Walker 1997). For example, in forests where *I. woodii* is dominant, tree seedlings may benefit from nutrient inputs through *I. woodii* leaf-litter or may be physically protected from herbivores by the taller *I. woodii* plants in addition to competing with it. Tree seedlings in these forests may therefore be subject to facilitative effects as well as subject to the combined effects of shading, below-ground competition from *I. woodii* and trees, and moisture stress.

Table 3. Analysis of covariance (ANCOVA) based on total biomass of tree seedlings as relative interaction index, and relative growth rate and root mass fraction between the *I. woodii* densities at Cape Vidal

Dependent variable	Source of variation	df	MS	*F*	*P*
Relative interaction index (biomass)	Species	1	0.02	0.63	0.433
	Density	1	0.11	2.92	0.095
	Residual	39	0.04		
Relative growth rate	Species	1	0.33	2.01	0.162
	Density	1	0.15	0.89	0.348
	Residual	59	0.09		
Shoot mass fraction	Species	1	0.18	57.77	<0.001
	Density	1	0.02	5.41	0.02
	Residual	59	0.003		

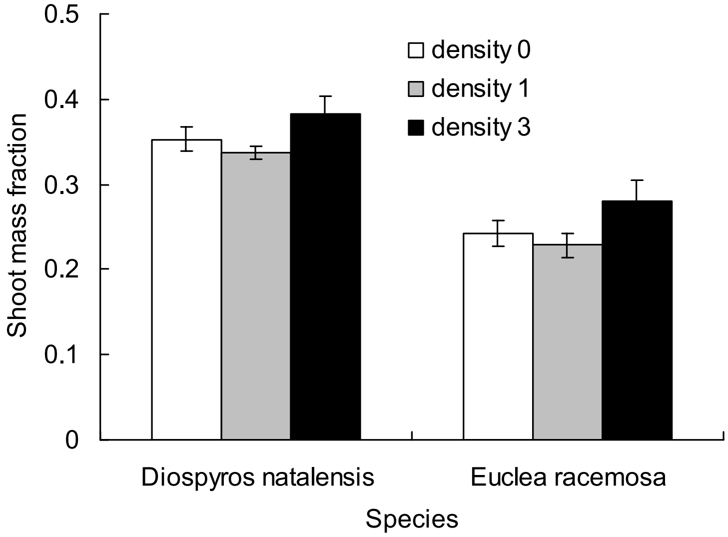

Fig. 6. Shoot mass fraction (mean ± 1SE) of *D. natalensis* and *E. racemosa* seedlings grown at three densities of *I. woodii* at Cape Vidal.

Water stress may exacerbate the competitive effect of understorey plants on tree seedlings. Some studies (e.g. Davies *et al*. 1998) have shown that seedling establishment success is diminished when moisture stress increases competition between tree seedlings and herbaceous vegetation. In coastal dune forests, the low water-holding capacity of soil may result in periodic water deficits (Venter 1976) whose effects on tree seedlings may be compounded during prolonged dry spells. Callaway *et al*. (1991) found increased competition for soil water between understorey plants and canopy trees when the trees had a high density of fine root biomass in the upper soil horizons that did not reach the water table. Similarly, *I. woodii* maintains a high density of fine root biomass in the upper soil horizons which suggests limited access to the water table, and may result in greater demand for moisture between the herb and tree seedlings. For shade tolerant species, the severity of moisture stress due to competition with understorey plants will be amplified by trait trade-offs between drought and shade tolerance (Huston 1994; Holmgren *et al*. 1997; Ninemets & Valladares 2006). The dominance of shade tolerant species in these forests (Chapter 7) may therefore have arisen during wetter periods in the past when droughts were rare and infrequent.

Reduction of biomass with competition was similar across the four tree species, resulting in no hierarchy of competitive ability among the tree species at either density of *I. woodii*. This outcome of competition in a forest understorey dominated by a monocarpic herb is not surprising. The life cycle of *I. woodii* consists of several years of vegetative growth followed by mass reproduction and synchronised mortality of the plants. During the middle and late stages of reproduction when *I. woodii* sets fruits and begins to disperse seed, the plant sheds its leaves and the understorey opens up, increasing the light reaching seedlings on the forest floor. Further increases in light conditions at the forest floor may occur as the adult plants die after seed dispersal, which increases the growth of seedlings of shade tolerant tree species in the forest. The relatively long periods of *I. woodii* cover and shading of the forest floor may have selected for shade tolerant species, with a gradual impoverishment or complete loss of light demanding species. This habitat filtering model posits that the environment allows tree species with a particular adaptation into the community, and excludes species without the attribute (Weiher & Keddy, 1999; Sargent & Ackerly, 2007), a scenario that may lead to the evolution of species that are functionally equivalent in competitive ability (see Chapter 7).

Finally, we note the major criticisms that have been leveled against pairwise experimental designs, particularly their assumption that interactions in diverse plant communities are the sum of pairwise species relationships (Dormann & Roxburgh 2005; Weigelt *et al*. 2007). However, our study system consisted of one dominant understorey species whose effects on individual tree species' seedlings may be more pervasive than any direct effects that tree seedlings will have on each other. Moreover, the widespread occurrence of seedlings of a species such as *D. natalensis* in these forests (Venter 1976; Nzunda *et al*. 2007) indicates that a particular seedling may have neighbourhoods that consist more of conspecifics and *I. woodii* than seedlings of other species. Interactions between conspecific tree seedlings and between tree seedlings and *I. woodii* may therefore be more important than seedling-to-seedling interactions among different species. In addition, Fowler (1982) reported successful prediction of community dynamics from pairwise species interactions. We conclude that recruitment limitation in these forests (Griffiths *et al*. 2007) and similar competitive responses to *I. woodii* of tree species may prevent competitive exclusion. This leads to coexistence and maintenance of species diversity in subtropical coastal dune forest.

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CHAPTER 7

PHOTOSYNTHETIC RESPONSES AND RELATIVE SHADE TOLERANCE OF COASTAL DUNE FOREST TREE SEEDLINGS IN RESPONSE TO A SUPPRESSIVE HERB

This chapter has been formatted for submission to *Journal of Ecology*.

Summary

1. The regeneration niche is commonly partitioned into shade tolerant and intolerant species to explain plant community assembly in forests. We examine the shade tolerance of tree seedlings in a subtropical coastal forest to determine whether the ecological filtering effect of a dominant, but synchronously monocarpic herb stratum, selects for species at either end of the light response continuum during the herb's vegetative and reproductive phases.

2. The photosynthetic characteristics of seedlings of 20 common tree species and the dominant semi-woody herb (*Isoglossa woodii*) were measured. Species were selected using abundance data from 19 experimental plots subdivided into subplots where the herb stratum was either intact or removed.

3. Seedlings were grown under greenhouse conditions at 12-14 % irradiance, and the light compensation point of five individuals per species measured using an open-flow gas exchange system.

4. Seedling abundance and species diversity were greater in plots without the herb, where light quality and quantity were greater. Average species richness increased from six in the intact understorey to 13 where the understorey was removed. Species had a narrow range of light compensation points from 2.1 ± 0.8 in *Celtis africana* to 6.4 ± 0.7 μmol m^{-2} s^{-1} in *Allophylus natalensis*. Mean apparent quantum yield at 0.08 mol CO_2 mol^{-1} was invariant among species. Rates of dark respiration were significantly lower in the generalist pioneer species *Acacia karroo* than in the forest pioneer *C. africana,* or other later successional phase forest species – *Mystroxylon aethiopicum, Strychnos gerrardii* and *Drypetes natalensis*.

5. *Synthesis*. Seedlings of most species were relatively shade tolerant, which may be a response to prevailing low light levels beneath the herb understorey. This phenotypic clustering of shade tolerance in many species from several families suggests that *I. woodii* imposes an environmental filter causing the loss of most light-demanding species; we reject the alternative hypothesis of evolutionary convergence of photosynthetic traits because the tree communities of these coastal forests constitute rather transient associations. Low intensity and frequency of disturbances, coupled with the prevention of competitive exclusion by recruitment limitation imposed by the herb stratum, probably maintain the phenotypic clustering of shade tolerant species in coastal dune forest. These data provide further support for the importance of influences on the seedling phase in determining forest tree dynamics.

Keywords: apparent quantum yield, dark respiration, habitat filter, *Isoglossa woodii,* light compensation point, light response curve, maximum assimilation, phenotypic clustering, shade adaptation.

Introduction

The effect of overstorey plants on light regimes in the forest understorey and on the forest floor has relevance to several competing theories of species coexistence. Theories based on chance as opposed to deterministic establishment of seedlings invoke different roles for light. Where species are recruitment or dispersal limited and establish at random, low light conditions on the forest floor act as a habitat filter causing phenotypic clustering towards shade tolerance so that this becomes a neutral trait among species (Hubbell 2005, 2006). In contrast, where species are deterministically selected according to niche requirements, establishment will be dependent on the spatially and temporally variable light regimes on the forest floor. For example, species with strong colonizing ability may be less shade tolerant and preferentially establish under higher light conditions in forest gaps (Denslow 1987; Schnitzer & Carson 2001) while shade tolerant species may readily establish on the shaded forest floor beneath the understorey. In this study we use photosynthetic traits to explore patterns of shade tolerance among tree species in a subtropical forest characterized by the suppressive herb *Isoglossa woodii* in the understorey. Photosynthetic light responses can provide important information about the establishment potential of seedlings in different environments and acclimation to changing conditions (Scholes *et al.* 1997; Lusk *et al.* 2008).

In tropical and temperate forests the low (< 1 % to 4 %) quantity of photosynthetically active radiation (PAR) reaching seedlings on the forest floor (Chazdon 1986) is often accompanied by up to 90 % reductions in the R:FR ratio or quality of light (Horsley 1993). A reduced quantity and quality of light curtails photosynthesis, net carbon gain, and growth of plants (Lambers *et al.* 1998). Based on their regenerative responses under impoverished light conditions beneath forest canopies, non-pioneer forest tree species are often classified as either shade tolerant or light demanding species (Chazdon *et al.* 1996; Whitmore 1996). Seeds and seedlings of shade tolerant species can germinate and grow successfully in the low light environment of the understorey, although their growth and survival are generally enhanced in higher light conditions (Brown *et al.* 1999; Busing & Brokaw 2002). For non-pioneer light demanding species, germination and successful establishment of seedlings are dependent on gaps in the overstorey, typically caused by disturbance (Brokaw 1985; Runkle 1998; Schnitzer & Carson 2001). The formation of a canopy gap for example, dramatically changes the light environment in the understorey, potentially increasing the photosynthetic carbon gain of seedlings of light demanding species and shade tolerant species too.

Although forests generally contain a continuum of light environments with arbitrary subgroups of species that vary little in shade tolerance (Swaine & Whitmore 1988; Osunkoya *et al.* 1994; Condit *et al.* 1996), the categorisation of light regimes between gap and understorey environments suggests that in general, for recruits or seedlings, forests consist of areas of relatively high and low light, respectively.

The understorey of Indian Ocean coastal dune forests of southern Africa is dominated by a monocarpic leafy and semi-woody herb, *Isoglossa woodii* (Acanthaceae).

The species shades out the forest floor for several years, after which it flowers synchronously and then dies back (Van Steenis 1978). Because of its dominance in the forest understorey, *I. woodii* reduces light levels over 65-95 % of the forest floor to < 2 % of ambient (Griffiths *et al.* 2007). The dieback of *I. woodii* changes the structure of the forest and may release tree seedlings from the effects of above- and below-ground competition for light and nutrients, respectively. *I. woodii* regenerates from seed immediately following the dieback period. Thus, there is a limited window of opportunity for light demanding tree species to recruit during the establishment of *I. woodii* seedlings. For shade tolerant species, however, recruitment may occur during the long vegetative phase of *I. woodii*. *Isoglossa woodii* therefore provides a temporal and spatial dichotomy of light environments in the understorey. We investigate whether there is a corresponding dichotomy of shade-tolerances among seedlings of tree species that commonly occur in a subtropical dune forest.

We hypothesise that *I. woodii* acts as an ecological filter selecting for species at either end of the light strategy continuum by facilitating recruitment of fast-growing, sun-adapted species whose seedlings can establish during the dieback period, while imposing strong selection for shade tolerant species able to establish and survive in the intervening years. Shade-adapted species tend to grow slowly but maintain high survivorship in the understorey, while light demanding non-pioneer species attain higher growth rates and low survivorship in shade and well-lit environments (Poorter & Arets 2003; Hubbell 2005). Shade adapted species therefore trade-off growth for increased survivorship, while light demanding species do the reverse, increasing growth at the expense of survivorship. The growth-versus-survival trade-off in shade adapted species is apparent in photosynthetic traits that result in maintenance of a positive leaf-level carbon balance in the low light conditions, such as lower dark respiration rates and low light compensation points (Sims & Pearcy 1991; Kitajima 1994; Lambers *et al.* 1998; Lusk 2002; Craine & Reich 2005). These photosynthetic traits increase survivorship but do not permit high growth rates. In contrast, photosynthetic traits for sun-adapted species include high photosynthetic capacities with correspondingly high respiratory costs, and high light compensation points (Sims & Pearcy 1991; Kitajima 1994; Kobe 1997; Lambers *et al.* 1998; Lusk 2002; Craine & Reich 2005). In addition, seedlings of shade tolerant species may maintain greater reserves of carbohydrates compared to light demanding species, thereby increasing their ability to buffer stress and increasing survivorship (Kobe 1997).

We predict that (1) the longer periods of diminished light levels beneath *I. woodii* will provide a regeneration niche for many shade tolerant species; and (2) the short period of increased light levels during the dieback of *I. woodii* will provide a niche for a few light demanding species. The light demanding seedlings that recruit during the die back period will belong to species that either maintain a seed bank in the soil or produce seed at about the same time as the masting of *I. woodii*.

Materials and Methods

Field surveys

Field surveys were carried out in the coastal dune forest at Cape Vidal, in the iSimangaliso Wetland Park (formerly Greater St. Lucia Wetland Park), KwaZulu-Natal, South Africa (28˚16' S, 32˚29' E). The site has a subtropical climate with a mean annual precipitation of approximately 1200 mm and a mean annual temperature of 21 ˚C (Schulze *et al*. 1997). The forest occupies sand dunes that extend from sea level to more than 100 m above sea level. The dominant canopy tree species include *Diospyros natalensis* (Harv.) Brenan, *Dovyalis longispina* (Harv.)Warb., *Euclea racemosa* Murray, *Drypetes natalensis* (Harv.) Hutch., *Sideroxylon inerme* L. and *Mimusops caffra* E. Mey. ex A.DC. (Nzunda *et al*. 2007a). The understorey is dominated by *Isoglossa woodii* C.B. Clarke, which covers from 65 % to 95 % of the forest floor (Griffiths *et al*. 2007).

The seedling community was surveyed in 19 10 m × 5 m experimental plots which were fenced to exclude herbivory by large mammals. Plots were subdivided into two 5 m × 5 m sub-plots, one of which had natural cover of *I. woodii* in the understorey and in the other the *I. woodii* cover was removed. In each sub-plot, five 1 m × 1 m quadrats were used to count the numbers of seedlings of each species in 2006. All plots were established in 2000, directly following the last *I. woodii* flowering event.

During April and May 2006, the understorey light environment within each quadrat in the herbivore-exclusion plots was examined by simultaneously measuring red (660 nm) and far-red (730 nm) transmittance through the *I. woodii* canopy using two

Apogee point sensors mounted side-by-side (Model QSO, Apogee Instruments), as well as the photosynthetically active radiation (400-700 nm; PAR) or photosynthetic photon flux density (PFD) in µmol m^{-2} s^{-1} using an Apogee quantum sensor. Red to far-red light is probably the most important spectral range of light for plants because of its strong effect on the phytochrome (Smith 1982), which in turn mediates many developmental responses of plants to light (Smith 1975) and is thus a measure of light quality. We used the 2006 survey data of herbivore-exclusion plots to relate seedling abundance to light availability in the forest understorey. The design of the plots allowed us to identify light-demanding from shade-tolerant species without the confounding effects of large mammalian herbivores.

Light response measurements

Common coastal dune forest tree species from Cape Vidal were measured. We selected the 18 most common species in terms of both tree and seedling abundance, apart from *Diospyros inhacaensis* F. White and *Olea capensis* L. which were abundant as trees but whose seedlings were not available when our sample was collected. These were replaced with *Apodytes dimidiata* E.Mey ex Arn. and *Acokanthera oppositifolia* (Lam.) Codd; two rare species that occupy overstorey and midstorey positions in the tree canopy (Table 1; Nzunda *et al.* 2007b). Five seedlings from each of these species were collected and transplanted into a standard potting soil mix in 1L pots. Seedlings were grown in a greenhouse at 12-14 % irradiance and moderated daytime temperature of 22-25 ˚C. A higher level of irradiance than is typical at the forest floor was used to ensure that seedlings produced some new leaves after potting and survived to the measurement period, but also reduces the likelihood of the seedlings adjusting their light response to low light conditions. For *I. woodii*, ‘seedlings’ were grown from shoots collected from five plants from Cape Vidal. To ensure that plants had recovered from transplantation shock before photosynthetic measurements were carried out, seedlings and *I. woodii* shoots were grown for at least 3 months and 2 months, respectively, before measurement.

Light response curves are useful in determining shade tolerance because they provide measures of dark respiration rate, the light compensation point, apparent quantum yields, and the maximum photosynthesis rates. Light response measurements were performed using an open-flow gas exchange measurement system (LI-6400, Li-Cor Inc., Lincoln, Nebraska, USA). All seedlings were approximately 30 cm in height when measured. Seedlings grown in the greenhouse were brought into the laboratory the day before the experiments and kept well watered. All measurements were made during the natural photoperiod (10h00 to 15h00) to ensure that photosynthesis had been naturally induced before the experiments began. From each individual, one fully expanded, attached leaf was sealed into the leaf chamber. The leaf chamber enclosed a 6 cm^2 area of leaf tissue, except in the case of species with compound or small leaves such as *Acacia karroo* Hayne and *Diospyros natalensis* when < 6 cm^2 of leaf tissue was enclosed. Air was passed through the chamber at a constant flow rate of 400 $\mu mol\ s^{-1}$ and a CO_2 concentration of 400 $\mu mol\ mol^{-1}$. Humidity was adjusted manually by passing the incoming air through a desiccant tube, which allowed leaf-to-air vapour pressure difference (VPD) to be held constant at 1 kPa. Leaf temperature was maintained at 20°C to match the average temperature at Cape Vidal.

Photosynthetically active radiation (PAR) was controlled using a red-blue light emitting diode light source. The leaf was exposed initially to a PAR of 200 $\mu mol\ m^{-2}\ s^{-1}$ and allowed to reach steady-state fluxes of CO_2 and H_2O, which took about 30min. After the leaf was acclimatized, irradiance was decreased in a stepwise fashion and we took three measurements of photosynthesis at 120s intervals at each of the following light levels: 200, 150, 100, 80, 60, 40, 30, 20, 10, and 0 $\mu mol\ m^{-2}\ s^{-1}$. We began measurements at the highest PAR because it has been shown that beginning at low irradiances results in limitation of photosynthesis at high irradiances due to insufficient opening of the stomata (Singsaas *et al.* 2001). The data from each plant were fitted to the monomolecular model (Causton & Dale 1990) using SPSS v. 15 (SPSS 2007). From the fitted data we developed light response curves, estimated the light saturated photosynthetic rate, determined the apparent quantum yield (the initial slope of the curve, Φ) as $a \cdot c \cdot e^{b}$, dark respiration rate as $a \cdot (1- e^{b})$ and calculated the light compensation point (LCP) for each individual using the ratio b/c, where a, b and c are fitted parameters of the monomolecular function $y = a \cdot (1-e^{b-c \cdot x})$ (Causton & Dale 1990). The initial slope of the photosynthesis light response curve represents the maximum efficiency of photochemical use of light in CO_2 fixation, and is a measure of the photosynthetic capacity when PAR is limiting (Long & Drake 1991). A seedling's LCP was only selected for use if its fit to the monomolecular function had a $R^2 > 0.9$. The LCP, taken as a mean of four to five individuals per species, was then compared among species as an indication of relative shade tolerance.

Table 1. Species used in the study including their position in the canopy, contribution (%) to total stem basal area in coastal dune forest, seedling abundance (as percentage of total woody seedlings) in the 2006 plot survey of both fenced and unfenced subplots, and seedling density. Nomenclature follows Coates Palgrave (2002) except for *Isoglossa woodii* when it follows Arnold & de Wet (1993)

Species	Family	Canopy position	% tree basal area	% abundance	*Seedling density per $4m^2$ (mean ± SD)
†*Isoglossa woodii*	Acanthaceae	Understorey	-	-	268 (502)
Teclea gerrardii	Rutaceae	Midstorey	3.65	32.22	6.65 (8.74)
Diospyros natalensis	Ebenaceae	Overstorey	11.98	28.16	9.99 (8.20)
Celtis africana	Celtidaceae	Overstorey	5.05	6.66	0.72 (1.57)
Allophylus natalensis	Sapindaceae	Mid-Overstorey	0.32	3.69	-
Mimusops caffra	Sapotaceae	Overstorey	10.64	3.60	1.76 (2.36)
Clausena anisata	Rutaceae	Midstorey	0.26	3.22	2.11 (5.36)
Scolopia zeyheri	Flacourtiaceae	Overstorey	2.36	2.39	-
Grewia caffra	Tiliaceae	Midstorey	0.33	2.08	-
Ochna natalitia	Ochnaceae	Mid-Overstorey	2.97	1.35	0.51 (0.90)
Sideroxylon inerme	Sapotaceae	Overstorey	8.09	0.98	0.10 (0.42)
Acacia karroo	Fabaceae	Overstorey	1.94	0.59	0.11 (0.36)
Dovyalis longispina	Flacourtiaceae	Mid-Overstorey	3.96	0.48	0.18 (0.59)
Euclea racemosa	Ebenaceae	Mid-Overstorey	4.46	0.37	0.26 (0.56)
Drypetes natalensis	Euphorbiaceae	Mid-Overstorey	5.02	0.30	0.60 (1.16)
Ziziphus mucronata	Rhamnaceae	Overstorey	5.72	0.30	0.03 (0.17)
Eugenia woodii	Mrytaceae	Overstorey	3.59	0.30	-
Strychnos gerrardii	Strychnaceae	Overstorey	8.88	0.23	0.56 (1.64)
Apodytes dimidiata	Icacinaceae	Overstorey	1.19	0.04	-
Mystroxylon aethiopicum	Celastraceae	Midstorey	1.42	-	-
Acokanthera oppositifolia	Apocynaceae	Midstorey	0.06	-	-

*Source: Table 1 in Nzunda *et al*. (2007b).

†Density of *I. woodii* based on 65 plots of 1m ×1m carried out in July 2007 during the seedfall period of the species' reproductive cycle.

Data analysis

To avoid Type I errors that may arise from testing null hypotheses on several correlated variables, differences between the *Isoglossa* treatments (removed and intact) were tested simultaneously in the dependent variables PAR, R:FR light, seedling abundance and species richness using MANOVA. The test statistic Wilks' lambda (likelihood ratio criterion) varies from 0 to 1 and tests the overall significance of the effects of the treatments on the dependent variables. If this test is significant, the tests of between-subjects effects are then consulted to identify responses contributing to the overall significant effect. Species richness and PAR were log- and square-root transformed, respectively, before analysis to ensure that variances were homogeneous between treatments.

Light saturated photosynthetic rates, light compensation points, apparent quantum yields and rates of dark respiration were compared among species using one-factor model I ANOVA, where each species was represented by the seedlings used to measure the photosynthetic light response curve. Relationships between seedling abundance, light quality and quantity, and light response parameters were explored using bivariate correlation. All data were analysed in SPSS v. 15 (SPSS 2007). Unless otherwise stated, we present results as the mean ±1standard error (s.e).

Results

Field surveys

Light quality and quantity, density and species richness of seedlings varied between the *Isoglossa* habitats (Wilks' $\lambda = 0.08$, $F_{4,29} = 82.89$, $P < 0.005$, Table 2). Both light quality and quantity increased with removal of *I. woodii*. There were also more species of tree in the *Isoglossa*-removed than in the *Isoglossa*-intact plots (Table 2). Tree species in the *Isoglossa*-intact plots were a subset of those in the *Isoglossa*-removed plots. Species richness and seedling density both increased with increasing light quality ($r = 0.73$ for richness and 0.62 for abundance, n = 34, $P < 0.001$). Species richness and seedling density also increased with increasing light quantity ($r = 0.63$, n = 34, $P < 0.001$ for richness; $r = 0.49$, n = 34, $P = 0.003$ for abundance).

Table 2. Values of four habitat parameters between *Isoglossa*-removed and *Isoglossa*-intact sites (MANOVA: Wilks' $\lambda = 0.080$, $F_{4,29} = 82.894$, $P < 0.0005$). Each parameter differed significantly between *Isoglossa* habitats ($F_{1,32} > 22.0$, $P < 0.0005$)

Variable	*Isoglossa*-removed	*Isoglossa*-intact
PAR (%)	6.1 ± 1.1	1.1 ± 0.1
R:FR ratio	0.8 ± 0.01	0.6 ± 0.01
Number of seedlings in 5m^2	108.8 ± 9.6	44.1 ± 9.5
Number of species in 5m^2	12.9 ± 0.7	5.8 ± 0.3

Seedlings were broadly clustered into groups of species which increased in density after removal of *I. woodii* (e.g., *Scolopia zeyheri* (Nees) Harv., *Celtis africana* N.L.Burm., above the diagonal on Fig. 1) and species that decreased after *Isoglossa* removal (e.g., *Clausena anisata* (Willd.) Hook.f. ex Benth., *Drypetes natalensis* (Harv.) Hutch., below the diagonal), and a third group made up solely of *Ochna natalitia* (Meisn.) Walp. whose density did not change after *Isoglossa* removal (species with densities lying on the diagonal, Fig. 1). Species that occur above and below the diagonal may correspond to the light demanding and shade tolerant guilds, respectively.

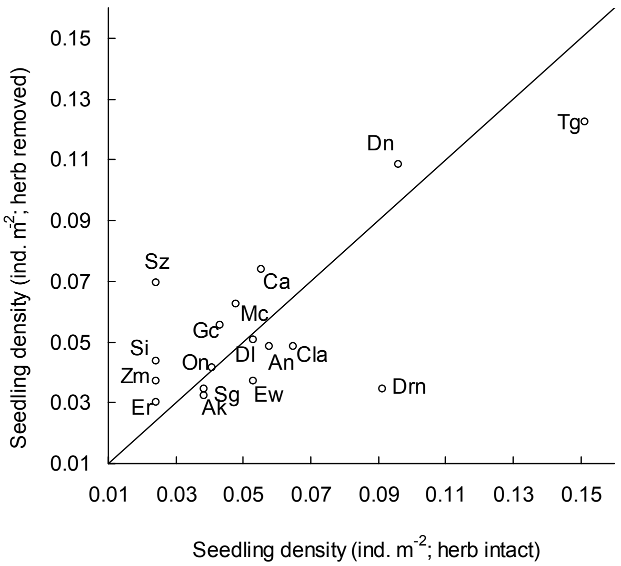

Fig. 1. Proportional seedling density in *Isoglossa woodii* intact (*Iw*+) and removed (*Iw*-) plots. Species names are *Acacia karroo* (Ak), *Allophylus natalensis* (An), *Celtis africana* (Ca), *Clausena anisata* (Cla), *Diospyros natalensis* (Dn), *Dovyalis longispina* (Dl) *Drypetes natalensis* (Drn), *Euclea racemosa* (Er), *Eugenia woodii* (Ew), *Grewia caffra* (Gc), *Mimusops caffra* (Mc), *Ochna natalitia* (On), *Scolopia zeyheri* (Sz), *Sideroxylon inerme* (Si), *Strychnos gerrardii* (Sg), *Teclea gerrardii* (Tg), and *Ziziphus mucronata* (Zm). *Acokanthera oppositifolia*, *Apodytes dimidiata* and *Mystroxylon aethiopicum* were absent in both *Isoglossa*-removed and intact plots.

Light response measurements

Maximum photosynthetic rates ($A_{\max}$) were attained at high PAR levels and varied among species ($F_{20,80} = 6.41$, $P < 0.001$). $A_{\max}$ was highest for *Acacia karroo* Hayne, *Grewia caffra* Meisn. and *I. woodii* (Table 3). *D. longispina, E. racemosa* and *Mystroxylon aethiopicum* (Thun.) Loes. had low maximum photosynthetic rates, with the lowest maximum assimilation of 1.63 ± 0.16 μmol m^{-2} s^{-1} measured for *Drypetes natalensis*, a midstorey species (Table 3). For a few species (*Diospyros natalensis, E. racemosa* and *Teclea gerrardii* Verdoorn), a two-fold increase in PAR from 100 to 200 μmol m^{-2} s^{-1} resulted in only a marginal increase in photosynthetic activity (Fig. 2). Response curves of the remaining species show that assimilation rates were still increasing with PAR levels beyond 200 µmol m^{-2} s^{-1}.

Light compensation points (LCP) were different among species ($F_{20,80} = 3.18$, $P < 0.001$), with the main difference between *C. africana* and each of *S. inerme, A. karroo, M. caffra, I. woodii* and *A. natalensis.* Light transmission beneath *I. woodii* (Table 2) represents a mean PFD of 4.2 ± 0.8 µmol m^{-2} s^{-1}, which is higher than the LCPs of *C. africana, O. natalitia* and *Diospyros natalensis* (Table 3), and suggests that seedlings of these species can obtain a positive carbon gain at the low light levels prevalent on the forest floor.

There was no relationship between the LCP of a species and the species' seedling abundance in either *Isoglossa*-intact ($r = 0.02$, n = 20, $P = 0.93$) or *Isoglossa*-removed plots ($r = -0.09$, n = 20, $P = 0.71$). A change in a species' seedling abundance after *Isoglossa* removal may reflect a species' light response traits such that species suppressed by the herb will increase in abundance after the herb is removed. The LCP was however, not related to the relative change in seedling abundance due to *Isoglossa* removal ($r = 0.22$, n = 17, $P = 0.39$), which was also evident in similar LCPs of species separated by the 1:1 diagonal in Fig.1 ($F_{2,17} = 0.29$, $P = 0.75$).

Apparent quantum yield was similar among species ($F_{20,80} = 1.53$, $P = 0.094$, Table 3). The rate of dark respiration varied among species ($F_{20,80} = 2.72$, $P = 0.001$), being lower in *A. karroo* than in *Strychnos gerrardii* N.E.Br. and *Drypetes natalensis* (Table 3). Dark respiration rate was also lower in *A. karroo* than in *C. africana, M. aethiopicum, S. gerrardii* and *Drypetes natalensis* ($F_{20,80} = 2.72$, $P = 0.001$). The LCP of species increased with dark respiration ($r = 0.89$, n = 21, $P < 0.001$) but was neither related to apparent quantum yield nor to maximum photosynthetic rate ($r = 0.328$ and -0.201, respectively; n =21, $P > 0.05$ in both cases, Fig. 3).

Table 3. Photosynthetic characteristics of *Isoglossa woodii* and seedlings of tree species from Cape Vidal. Mean values (±SE) are presented for light saturated photosynthetic rate, light compensation point, apparent quantum yield and dark respiration rate

Species	Light saturated photosynthetic rate (µmol m^{-2} s^{-1})	Light compensation point (µmol m^{-2} s^{-1})	Apparent Quantum Yield (mol CO_2 mol^{-1})	Dark respiration (µmol m^{-2} s^{-1})
Celtis africana	4.65 (0.69)	2.14 (0.84)	0.08 (0.01)	0.18 (0.08)
Ochna natalitia	3.73 (0.29)	2.59 (0.60)	0.08 (0.01)	0.21 (0.07)
Diospyros natalensis	2.98 (0.65)	2.78 (0.50)	0.06 (0.02)	0.18 (0.05)
Strychnos gerrardii	3.88 (0.44)	3.24 (0.72)	0.07 (0.01)	0.22 (0.04)
Drypetes natalensis	1.63 (0.16)	3.44 (0.34)	0.06 (0.00)	0.19 (0.03)
Scolopia zeyheri	5.37 (0.70)	3.52 (1.08)	0.08 (0.01)	0.28 (0.09)
Ziziphus mucronata	4.18 (0.34)	3.69 (0.47)	0.09 (0.00)	0.31 (0.05)
Eugenia woodii	4.42 (0.68)	3.75 (0.25)	0.09 (0.01)	0.31 (0.04)
Mystroxylon aethiopicum	2.74 (0.41)	4.24 (0.95)	0.05 (0.01)	0.20 (0.05)
Clausena anisata	4.80 (0.43)	4.29 (1.13)	0.07 (0.01)	0.30 (0.08)
Grewia caffra	6.00 (0.36)	4.37 (0.14)	0.08 (0.01)	0.33 (0.02)
Dovyalis longispina	3.95 (0.44)	4.53 (0.59)	0.08 (0.01)	0.37 (0.09)
Acokanthera oppositifolia	2.93 (0.59)	4.71 (0.30)	0.07 (0.00)	0.33 (0.02)
Apodytes dimidiata	5.21 (0.29)	4.82 (0.47)	0.09 (0.00)	0.40 (0.05)
Euclea racemosa	2.42 (0.54)	4.89 (0.91)	0.08 (0.00)	0.35 (0.07)
Teclea gerrardii	2.98 (0.32)	5.33 (0.53)	0.08 (0.00)	0.38 (0.03)
Sideroxylon inerme	3.39 (0.46)	5.83 (1.26)	0.08 (0.00)	0.45 (0.09)
Acacia karroo	7.42 (1.33)	5.95 (0.76)	0.10 (0.02)	0.59 (0.17)
Mimusopa caffra	4.71 (0.52)	6.16 (0.43)	0.09 (0.02)	0.54 (0.13)
Isoglossa woodii	5.72 (0.20)	6.28 (0.48)	0.08 (0.00)	0.50 (0.05)
Allophylus natalensis	4.40 (0.22)	6.36 (0.69)	0.07 (0.00)	0.41 (0.03)

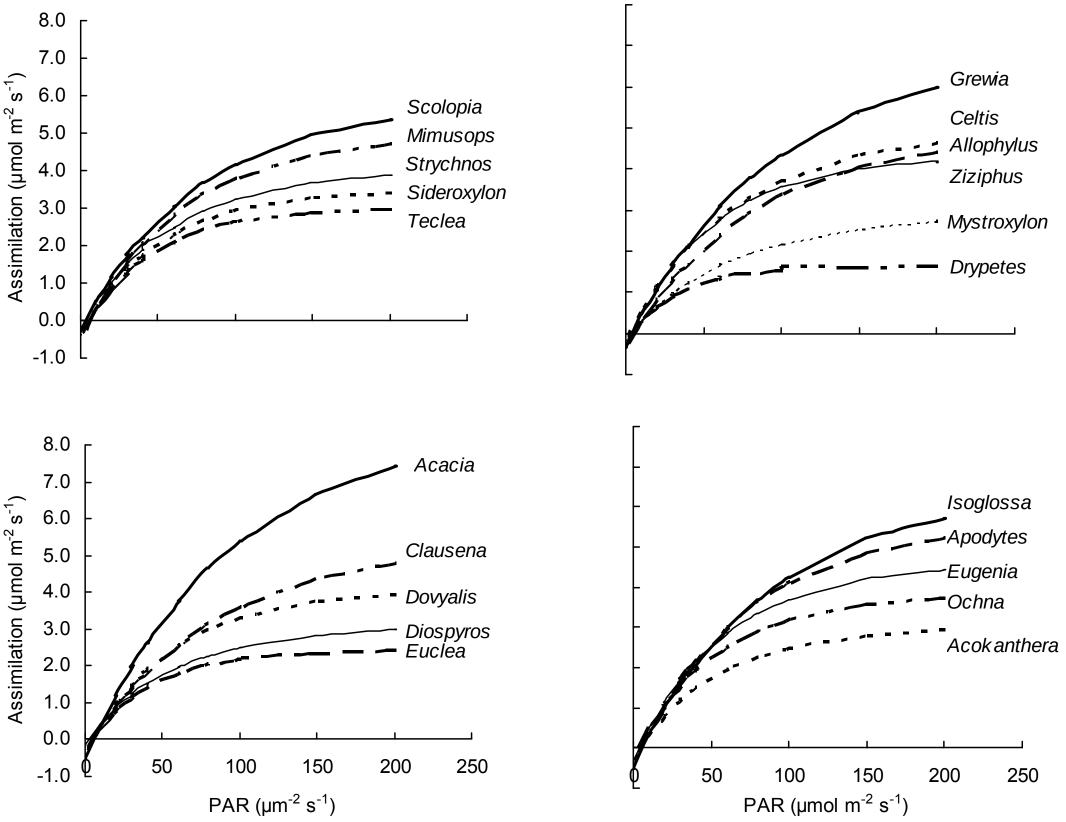

Fig. 2. Photosynthetic light response curves of *Isoglossa woodii* and seedlings of tree species from Cape Vidal grown in a greenhouse. Species (specified by genus) are grouped together for convenience.

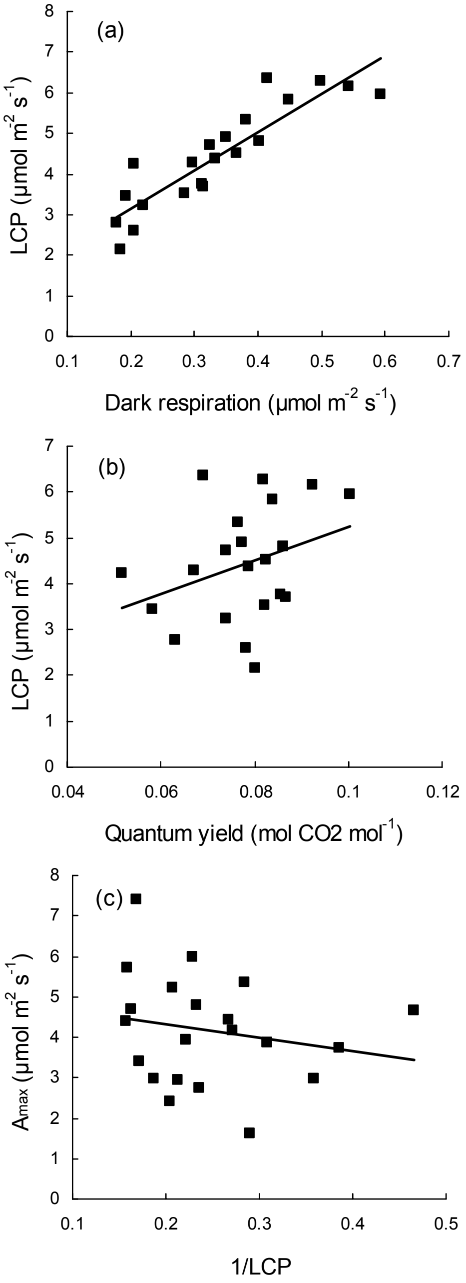

Fig. 3. Bivariate relationships between (a) LCP and dark respiration, (b) LCP and apparent quantum yield, and (c) inverse of LCP and $A_{\max}$. The positive relationship between LCP and dark respiration is expected: species with higher light compensation points have higher rates of dark respiration, while LCP and apparent quantum yield were not correlated, also as expected. The relationship between shade tolerance (1/LCP) and $A_{\max}$ was weaker than expected ($r = -0.201$, n = 21, $P = 0.383$): species with high $A_{\max}$ are shade-intolerant.

Discussion

Isoglossa woodii clearly affects the light environment, species composition and the density of tree seedlings on the forest floor. *I. woodii* reduced the spectral quality of light by 28 %, PAR transmittance by more than 80 %, and was associated with > 30 % reduction in species composition. Although the tree overstorey may reduce the light levels reaching the forest floor, the most pervasive effect on reduction of PAR levels is attributed to understorey vegetation (Montgomery & Chazdon 2001). The forest understorey plays a crucial role in determining tree recruitment and consequently forest structure and dynamics from temperate to tropical rainforests (George & Bazzaz 1999; Wang & Augspurger 2004; Coomes *et al*. 2005; LaFrankie & Saw 2005; Benítez-Malvido 2006). Diminished survivorship and growth of tree seedlings due to light attenuation by the understorey has been reported in many studies (George & Bazzaz 2003; Farris-Lopez *et al*. 2004; Slocum *et al*. 2004; Griffiths *et al*. 2007).

Various degrees of shade tolerance should be evident among tree species whose individuals spend a significant proportion of their vulnerable stages of growth in the shade. We show that the presence of *I. woodii* in the understorey is associated with > 50 % reduction of seedling density. However, the photosynthetic light response curves and light compensation points of the 21 species examined in this study show that shade tolerance is a feature of seedlings of all these species, including those that are poorly represented beneath *I. woodii.*

Our values of apparent quantum yield (Φ) were similar to those reported in other studies (Ehleringer & Björkman 1977; Long *et al*. 1993; Singsaas *et al*. 2001), and were relatively high as they were closer to the theoretical maximum of 0.111 for C_3 plants (Long *et al*. 1993). The high quantum yields of seedlings in this study show that these species have elevated photosynthetic capacities at low light levels, which is particularly important for plants that are exposed for most of the day to photon irradiances insufficient to meet the energetic and carbon requirements of growth and survival. High and constant quantum yields coupled with high sunfleck use efficiency increases seedling survival in the understorey (Long *et al*. 1993; Singsaas *et al*. 2000).

We expected the light compensation points of the 21 most important species in this forest to be highly variable but with a clear dichotomy of shade tolerant and shade intolerant species (Condit *et al*. 1996; Poorter & Arets 2003). This we did not find, but rather found low LCP values similar to the range of 1-5 µmol m^{-2} s^{-1} reported for understorey plants in temperate and tropical forests (Chazdon *et al*. 1996), which suggests that these species are shade tolerant. The light demanding functional group is therefore absent or is represented in the forest by only a few species, a finding that is consistent with the lack of a trade-off between A_{max} and shade tolerance (Fig. 3).

Isoglossa may have caused a dichotomy in the light response of species but because of its dominance and relatively long maturation time of 7 years (Griffiths *et al*. in press), the shaded micro-environment at the forest floor has selected against the light demanding cluster which has consequently been lost over time, while the relatively shade tolerant cluster has been favoured. This scenario explains (a) the lack of an apparent trade-off between LCP and A_{max} in the extant group of species, and (b) the comparatively low LCPs recorded from Cape Vidal. In addition, this conceptual framework offers the first insights into whether light response traits among tree species at Cape Vidal have arisen as a consequence of habitat (*Isoglossa*) filtering as opposed to trait convergence. In the habitat filtering model, the environment allows tree species with a particular adaptation into the community, and excludes species without the attribute (Weiher & Keddy 1999; Sargent & Ackerly 2007). This model predicts that the community will have species that have similar values for traits that are critical to the persistence of the species in their environment, described as trait/phenotypic clustering (Webb 2000; Webb *et al*. 2002; Cavender-Bares *et al*. 2004). As in the plant community at Cape Vidal, the species showing phenotypic clustering need not be phylogenetically related, but may have broadly equivalent ecological requirements. For example, Cavender-Bares *et al*. (2004) have shown that distantly related oak species in Florida tend to evolve towards convergence for similar habitat preferences while closely related species have more contrasting habitat requirements. Convergence of photosynthetic traits among tree species at Cape Vidal is unlikely because the species are distantly related from many different clades.

The nature of disturbances in the forest may determine the long term pre-dominance of shade tolerant over light demanding species. In subtropical coastal dune forest, disturbances are of low severity (Nzunda *et al*. 2007a,c). There is a low frequency of occurrence of large gaps, and together with the relatively long periods of *I. woodii* vegetative cover, this means that high light environments are rare both spatially and temporally. The absence or low numbers of light demanding species may therefore reflect the rarity of high light environments in the forest (Hubbell 2005). In fact, species with seed and seedlings already present may benefit from increased light conditions in canopy gaps and in understorey gaps created by the post-reproductive die back of *I. woodii*, which further reduces the potential for light demanding species to establish. This is particularly so for species showing continuous or annual seed production (Hartshorn 1978, 1980). *Diospyros natalensis, Sideroxylon inerme* and *Euclea racemosa* appear to produce large quantities of seed every year but it is only *D. natalensis* whose seedling densities mirror its prolific seed production (cf. Table 1 in Nzunda *et al*. 2007b). Seedlings of the important species may therefore dominate both in high and low light conditions in a way similar to that reported by Hubbell *et al*. (1999) on Barro Colorado Island in Panama, and by Webb & Peart (2000) at Gunung Palung in Borneo, Indonesia.

Niche assembly theory suggests that two competitively similar species cannot stably coexist (Loreau 2004; Hubbell 2005). Competitive exclusion should therefore be common in communities where functional equivalence of species has evolved through habitat filtering. There are however, only very few examples of competitive exclusion in the literature (Hubbell 2005). In a community where dispersal and recruitment limitation are strong, competitively inferior species may occupy establishment sites by default because the competitively superior species fail to reach these sites. Strong dispersal and recruitment limitation prevent competitive exclusion among functionally equivalent species of tropical rain forest communities (Hubbell *et al*. 1999; Hubbell 2005, 2006). In a seedling census of 95 1-m^2 quadrats located in 19 5 m × 5 m herbivore-exclusion plots beneath *I. woodii* in 2006, 44 % of the important tree species had no seedlings at all while 56 % (18 species) occurred in < 1 % of the plots (Z. Tsvuura unpublished data). Only 4 species (12.5%) occurred in more than half the plots. This suggests that the important species may be recruitment limited. Tsvuura *et al*. (2007) suggested that some degree of recruitment limitation occurs in coastal dune forests. If recruitment limitation is strong, then it provides a mechanism by which competitive exclusion is prevented, thereby maintaining species coexistence and the phenotypic clustering of shade tolerant species reported here.

We conclude that most species examined in this study are shade-tolerant. These species have low light compensation points, high quantum yield and low rates of dark respiration, which shows phenotypic clustering of shade-tolerant taxa. These features suggest adaptation to the ecological filter imposed by an *I. woodii* understorey beneath which their seedlings must establish.

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CHAPTER 8

THE EFFECTS OF *ISOGLOSSA WOODII* ON WOODY SEEDLING ESTABLISHMENT IN A COASTAL DUNE FOREST

Format follows *Ecological Monographs*

ABSTRACT

Understorey vegetation is often an ecological filter that shapes the density and species composition of the tree community in a forest. We examined the effect of a pervasive monocarpic woody herb *Isoglossa woodii* and associated herbivory on tree seedling emergence and establishment in a subtropical coastal dune forest. The effects of herbivores and competition by *I. woodii* were examined by sampling over a full reproductive cycle of the understorey species. Nineteen plots were established in 2000. Each plot measured 10 m × 10 m and contained four 5 m × 5 m sub-plots of which two were fenced to exclude large herbivores. The *I. woodii* stratum was removed from one of each pair of fenced and unfenced sub-plots, resulting in a fully factorial design with all possible combinations of fencing and *I. woodii* cover. Once a year (2001-2008) we measured the height, stem diameter and species composition of seedlings in five permanent 1 m^2 quadrats located in each half of the sub-plots. The height and stem density of the intact *I. woodii* layer in each sub-plot were also measured. Air temperature at 0.2 m above the ground was measured in the intact and cleared understorey of the fenced sub-plots. Soil samples were collected from all sub-plots for measurement of fertility (Ca, Cu, exchangeable acidity, Mg, Mn, pH, P, K, total C, total cations, Zn, and total N) and soil moisture during late 2005 into 2006 when sufficient time had passed for the *I. woodii* and herbivore treatments to have an effect. Soil parameters and *I. woodii* height and density were used as covariates in the seedling analysis. In 2004 we established 30 paired 10 m × 10 m plots located adjacent to each other in areas with understorey cover and areas with understorey gaps and measured tree seedling density and species richness in five 1 m^2 quadrats in each plot. The *I. woodii* and herbivore treatments had no effect on any of the measured soil parameters. The herb also had no effect on air temperature and soil moisture. The density and species composition of tree seedlings were greater in the cleared than beneath the intact understorey. Herbivory of woody seedlings was significantly greater in the cleared areas compared to beneath the intact understorey. The effect of the *I. woodii* understorey on reducing seedling density and species richness was greater than the effect of herbivory. The magnitude of these differences and effects increased with time. The effect of the *I. woodii* cover on plant functional types differed as well, with significantly reduced survival of the common pioneer species *Celtis africana* beneath *I. woodii*. In contrast, no difference in survival of a late successional shade-tolerant species *Diospyros natalensis* was detected. In the paired plots survey, the tree seedling community beneath understorey vegetation was a subset of that in understorey gaps, with greater seedling density and species richness in the gaps than beneath the understorey. We argue that *I. woodii* is a strong ecological filter that affects the diversity and establishment of woody seedlings and hence tree dynamics in subtropical dune forests. The herb filter combines several interacting mechanisms, but low light is the main limiting determinant of seedling establishment and survival in these forests. Gaps in *I. woodii* cover provide opportunities for regeneration of tree species that are sensitive to conditions associated with understorey cover. Further seedling establishment may be enhanced by monocarpic senescence of *I. woodii* at several-year intervals. *Isoglossa woodii* thus acts as a filter that selects for convergence in traits needed to cope with establishment conditions imposed by its dominance in the forest.

Key words: *ecological filter;* Isoglossa woodii*; moisture deficits; monocarpy; species composition; subtropical coastal dune forest; tree regeneration; seedling establishment.*

INTRODUCTION

The understorey stratum may act as a sieve selectively filtering certain species and genotypes so that the characteristics of surviving seedlings and saplings determine community structure and ecology (George and Bazzaz 1999a,b, 2003). Understorey effects on seedling establishment have been demonstrated in several studies. For example, Maguire and Forman (1983) have shown that understorey plants determine the density and distribution of seedlings of the main tree species in hemlock-hardwood forests in the eastern United States. In a Costa Rican rainforest, Denslow et al. (1991) demonstrated that an understorey dominated by palms and cyclanths influenced the distribution and abundance of establishing *Inga* seedlings. Similar effects of understorey vegetation have been demonstrated in *Nothofagus*-dominated forests in Chile (Veblen 1982, González et al. 2002), hardwood forests in the northeastern United States (Horsley 1993, George and Bazzaz 1999a,b, 2003), and mixed-hardwood forests of the southeastern United States (Clinton and Vose 1996, Nilssen et al. 1999, 2001). In this study we examine the effect of a pervasive monocarpic understorey herb, *Isoglossa woodii*, on seedling establishment in Indian Ocean coastal dune forest.

Understorey vegetation constrains seedling establishment in several indirect ways, such as by changing soil fertility and pH, and diminishing light levels through interception (Cross 1981, Chazdon 1986, Nakashizuka 1987, Horsley 1993, Clinton and Boring 1994, Nilsen et al. 2001) or directly through belowground competition, excretion of allelopathic compounds, exposure to pathogens transferred from the upper layers of the canopy, or mechanical damage resulting from falling phytomass (Clark and Clark 1989, Denslow et al. 1991, Nilssen et al. 1999, 2001, Coomes and Grubb 2000, Scariot 2000, Lei et al. 2002, Paul et al. 2004, Slocum et al. 2004, Nilsson and Wardle 2005). In addition, leaf litter beneath understorey plants may prevent seeds from reaching the soil and impede seedling germination and establishment (Facelli and Pickett 1991, Molofsky and Augspurger 1992, George and Bazzaz 1999a, Farris-Lopez et al. 2004). However, although understorey vegetation may suppress seedling establishment it can also facilitate the process (Callaway and Walker 1997; Gaxiola et al. 2008). For example, through hydraulic lift, deep-rooted understorey plants can absorb water from greater depths and release it to the upper drier layers where it is available for use by shallow-rooted plants such as tree seedlings (Dawson 1993, Caldwell et al. 1998, Horton and Hart 1998, Moreira et al. 2003).

The distribution of light on the forest floor is often categorised into very low (1-2 % of photosynthetically active radiation, PAR) light environments beneath the understorey stratum, and high (sometimes up to 35 %) light levels at the centre of large canopy gaps (Chazdon and Fetcher 1984). In addition to reduction in light levels by canopy trees, understorey vegetation further reduces the amount of light reaching the forest floor thereby suppressing tree seedlings (Horsley 1993, George and Bazzaz 2003, Farris-Lopez et al. 2004, Griffiths et al. 2007).

In addition to light attenuation, understorey plants may compete with tree seedlings (see Chapter 6, this study) for belowground resources such as soil moisture and nutrients (Clinton and Vose 1996, Nilsen et al. 2001). As tree seedlings are at a size disadvantage compared to understorey plants, the understorey plants are likely to uptake a disproportionately greater quantity of resources, a phenomenon described as “size-asymmetric competition” (*sensu* Schwinning and Weiner 1998). Size-asymmetric competition by the herb stratum may result in species-specific reductions in growth and survivorship of tree seedlings, thereby influencing the species composition and structure of the forest.

Understorey plants may further indirectly affect the establishment of tree seedlings by influencing the behaviour of seed and seedling predators. For example, dense understorey shrub cover may provide habitat to seed predators, which result in higher seed losses beneath the shrub cover (Wada 1993, Holl 2002). However, in lowland neotropical forest, seed and seedling predation may be higher in gaps than in the understorey because rodents use the fallen trees as shelter from their own predators (Schupp and Frost 1989). If seed and seedling predation are high, recruitment could be limited thereby inhibiting tree regeneration (Callaway 1992, Wada 1993, Hulme 1996).

Seedling herbivory by insects, rodents and large mammalian herbivores can have a profound effect on forest regeneration (Sork 1987, Augspurger and Kitajima 1992, McPherson 1993, Pastor et al. 1993, Struhsaker et al. 1996, Fuchs et al. 2000, Ickes et al. 2001, 2003, Boyes 2007, but see also Castleberry et al. 2000, and Collins 2003). For example, Boyes (2007) reported that succession of *Acacia karroo*-dominated stands to primary forest was arrested by herbivore pressure from large mammals at the same coastal dune forest site investigated in this study. Davidson (1993) found that insect and vertebrate herbivory can influence the trajectory of vegetation succession. The direct and indirect effects of herbivory may change the density and species composition of the seedling pool (Sork 1987, Liang and Seagle 2002, Howlett and Davidson 2003), which may ultimately change the species composition of the forest (Veblen et al. 1989, Molofsky and Fisher 1993). When large herbivores are selective browsers, their feeding may alter species dominance patterns resulting in changes to nutrient cycling (Pastor et al. 1993, Rooney and Waller 2003).

The objective of this study was to explore the effect of a clonally monodominant understorey species *Isoglossa woodii* (Acanthaceae), on tree seedling establishment and recruitment in an Indian Ocean subtropical coastal dune forest. We posit that the understorey acts as an ecological filter that shapes forest tree community composition and structure by altering the emergence and establishment of woody seedlings and thus tree regeneration (Denslow et al. 1991, George and Bazzaz 2003, Nilsson and Wardle 2005). *Isoglossa woodii* is a broad-leaved, semi-woody and robust herbaceous shrub that exhibits synchronous monocarpy at 4-7 year cycles (Van Steenis 1978). The importance of the monocarpic life-history strategy of the species is its profound influence on forest ecology by providing a major structural change in the forest every 4-7 years. The species may provide environmental conditions for unique regeneration strategies among tree species by promoting the establishment of seedlings of shade-tolerant species during the growth phases of *I. woodii*, and facilitating the establishment of light-demanding species during its die-back after reproduction. We monitored emergence and establishment of tree seedlings and measured tree seedling sizes, abundance and species composition over eight years in the Cape Vidal dune forest. Permanent sample plots were experimentally manipulated to separate the effects of understorey layer from the effects of herbivory by large mammals. We predict that: (i) removal of understorey vegetation increases both the density and species composition of woody seedlings; (ii) herbivore exclusion increases seedling density in both intact understorey and understorey gaps; and (iii) given the pervasive nature of the herb cover we expected the effect of the herb on seedling diversity and abundance to be greater than the effect of herbivory.

METHODS

Experimental design

To test the effects of *I. woodii* on woody seedling establishment in coastal dune forests, 19 permanent experimental plots were established at Cape Vidal in March 2001. Each plot measured 10 m × 10 m and contained four 5 m × 5 m sub-plots of which two were fenced to exclude large herbivores that are abundant at Cape Vidal (Boyes 2007) and test the effect of herbivory on both seedling and *I. woodii* growth and recruitment (Fig. 1). The two remaining sub-plots were not fenced. *Isoglossa woodii* was removed (*Isoglossa*-removed) from one of each pair of fenced and unfenced sub-plots and retained in the other sub-plot of each pair (*Isoglossa*-intact). At the start of the experiment the herb was 6 months of age, having established after a dieback event in late 2000. The herb was removed from sub-plots by cutting the stem at its base. There was no regeneration of the herb once pruned in this way and no further establishment from seed was recorded either. A different treatment was applied to each sub-plot. The experiment is based on a crossed two-factor repeated measures design (Fig. 1).

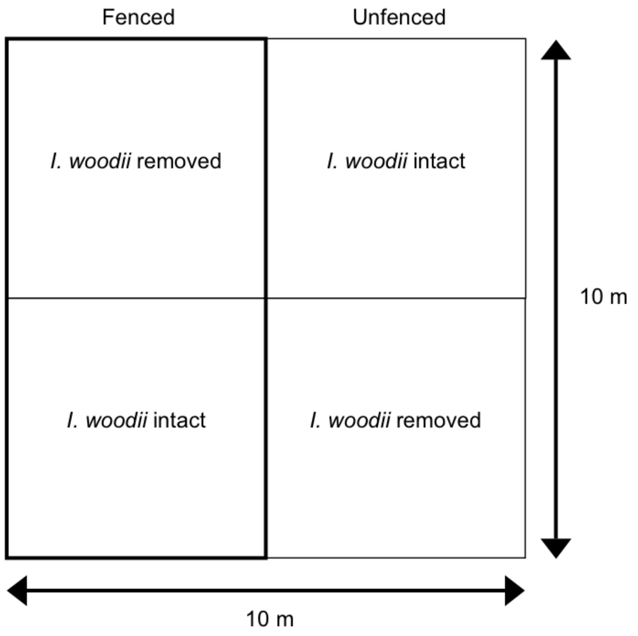

FIG. 1. Experimental plot design showing the layout of the *Isoglossa woodii* treatments in relation to the herbivory treatments.

Microsite conditions

Five years after the start of the experiment, in November 2006, we took soil samples to establish whether the herb and herbivore treatments affected the physico-chemical properties of the soil. Five sub-samples were collected from each sub-plot and pooled following standard soil sampling techniques in forests (Bélanger and van Rees 2008). Soil cores were collected to a depth of 6 cm as this is the root depth of the herb and most seedlings. They were analysed by the Soil Fertility and Analytical Services unit of the Cedara Agricultural College, KwaZulu-Natal, for clay (%), bulk density (g/mL), calcium (Ca, mg/L), copper (Cu, mg/L), exchangeable acidity (cmolc/L), magnesium (Mg, mg/L), manganese (Mn, mg/L), pH (KCl), phosphorous (P, mg/L), potassium (K, mg/L), total carbon (%), total cations (cmolc/L), zinc (Zn, mg/L), and total nitrogen (%).

Hydraulic lift can result in shallow-rooted plants growing in close proximity to deep-rooted plants benefiting from this association (Dawson 1993, 1996, Caldwell et al. 1998). To determine the relative effects of *Isoglossa woodii* on moisture content of superficial soils, we collected soil samples from the upper 5 cm of soil in the fenced *Isoglossa*-intact and *Isoglossa*-removed sub-plots during 2005 and 2006. Composite samples were obtained by combining five samples from each sub-plot. We determined gravimetric water content by drying composite samples at 105 °C until constant mass was attained.

We used temperature data loggers (DS1921 Thermocron iButtons) to measure temperature in the fenced (both the *I. woodii*-removed and *I. woodii*-intact) sub-plots. In each sub-plot, an iButton was attached to a wire stake and positioned at a height of 0.2 m above the ground. Temperature readings were obtained from each of the 38 sub-plots at one-hour intervals between February 2005 and February 2007.

Measurements of Isoglossa woodii *and tree seedlings*

The density and height of *I. woodii* was measured in five non-permanent 0.5 m × 0.5 m quadrats once a year (2001-2008) in the *I. woodii*-intact sub-plots. Once each year in late March or early April, we measured species composition, height and stem diameter of all tree seedlings (< 50 cm in 2001, but including 2001 seedlings that were > 50 cm in subsequent years) in five permanent 1 m × 1 m quadrats in each of the four sub-plots.

We supplemented the tree seedling data from the *I. woodii*-intact and -removed experiments using 30 paired plots measuring 10 m × 10 m that were established side-by-side in areas with and without *I. woodii* cover (understorey thickets and gaps respectively). Details of this experiment are given in Griffiths et al. (2007).

Tree seedling survivorship

We measured tree seedling survival by comparing seedling presence or absence in each 1 m × 1 m quadrat between selected years. We used data on tree seedling survival during 2003 and 2006, which represents two years after the dieback of *I. woodii* and one year before the onset of flowering of the species, respectively. Tree species were divided into two groups based on their importance values (important vs. not important) in the permanent plot surveys of 2003 and 2006, and the paired plots survey of 2004. Our importance value data on these species are consistent with the findings of another study in the same forest (Table 1 in Nzunda et al. 2007b). Seven species had high frequencies of occurrence and were classified as important, while nine species occurred in only some of the plots and were designated as less important species.

Data analysis

Soil fertility data were analysed in a two-way multivariate analysis of covariance (MANCOVA) using the general linear models (GLM) procedure, with herbivory (fenced and unfenced) and *Isoglossa* habitat (intact and removed) as fixed factors and soil bulk density as a covariate. To reduce redundancy in the MANCOVA analysis, we excluded from the analysis one of each pair of strongly correlated ($r > 0.95$) parameters, which lessens the impact of collinearity and reduces the dimensionality of the analysis thereby improving the robustness of the MANCOVA test (Quinn and Keough 2002). We performed univariate analysis of variance on each response variable separately before carrying out the MANCOVA analysis to check for normality of residuals and equality of variances. Two response variables (Cu and Zn) were square-root transformed to normalise residuals (Kéry and Hatfield 2003). No suitable transformation could be found for exchangeable acidity, which was excluded from the MANCOVA analysis and analysed separately using the non-parametric Friedman's test with plots as blocks and four treatment levels (*Isoglossa*-intact, herbivory; *Isoglossa*-intact, no herbivory; *Isoglossa*-removed, herbivory, *Isoglossa*-removed, no herbivory).

Gravimetric water content data were analysed using a one-way repeated measures analysis of covariance (ANCOVA) with *I. woodii* height and stem density in each sub-plot each year as covariates. Analysis of variance with time (10h00, 12h00 and 14h00) as a block was used to compare mean monthly air temperature between the *Isogossa*-intact and *Isogossa*-removed plots.

Seedling density and species richness data were analysed by a two-factor repeated measures design using the GLM procedure. Herbivory and *Isoglossa* habitat were the main fixed factors with plots as blocks and time (2001-2008) as the repeated measure. For seedling density, Plot 18 in 2008 was considered an extreme outlier because of large numbers of *Scolopia zeyheri* seedlings (325 m^{-2}) that germinated the previous year in the unfenced, *Isoglossa*-removed treatment. Seedling density was therefore analysed twice: with and without the data from plot 18. Height and density of *I. woodii* measured each year were used as covariates in the analyses. These variables were strongly correlated ($0.36 < r < 0.99$, $P < 0.02$). To reduce problems of collinearity, we used principal components analysis (PCA) with varimax rotation (Quinn and Keough 2002) to identify composite variables of height and density of *I. woodii* for inclusion as covariates in the repeated measures analysis of variance. Non-significant covariates were removed from the model to increase the power of the test.

To determine which environmental variables influence density and species richness of seedlings, the relationship between seedling density and diversity and each of several soil fertility variables was explored using principal components analysis (PCA) to reduce the number of influential variables, and then by generalised linear models to determine the relative importance of each of the selected variables. Seedling data were from fenced plots from which *Isoglossa* had been removed. This enabled the effect on seedling density and diversity of soil fertility alone to be examined. The bulk density of the soil, total C, total N, total cations, P, K, Ca, Mg, pH, exchangeable acidity, Zn, Mn, clay, Cu, gravimetric water content and soil organic matter were all examined in PCA for their orthogonality. Because the original data were not measured on the same scale they were standardised by use of the correlation matrix as opposed to the variance-covariance matrix (James and McCulloch 1990). Generalised linear model fits to the data comprising the selected soil fertility variables were conducted against seedling diversity and density separately, based on a Poisson distribution and log-link function, and normal distribution and identity link function, respectively. Model selection was conducted in an information theoretic framework using Akaike's Information Criterion (AIC). As seedling density and species richness between consecutive years were highly correlated, we used only the 2006 data, which was also the time that soil moisture and fertility variables were measured. All analyses were carried out using GENSTAT 11.1 (Lawes Agricultural Trust 2008) statistical package.

RESULTS

Microsite conditions

The potential effects of herbivory on tree seedlings was not associated with any of the measured soil parameters: N, P, K, Mg, Mn, Ca, Cu, Zn, pH, total carbon, and total cations (MANCOVA: Pillai's trace = 0.013, $F_{3,\ 69}$ = 0.304, P = 0.822). Similarly, *Isoglossa woodii* cover was not associated with these soil properties (Pillai's trace = 0.052, $F_{3,\ 69}$ = 1.251, P = 0.298). The interaction between *I. woodii* cover and herbivory was also not significant (Pillai's trace = 0.036, $F_{3,\ 69}$ = 0.87, P = 0.461). There was no association between the *I. woodii*-herbivory treatments and exchangeable acidity (Friedman's Chi-square = 0.87, P = 0.818). The mean value of each soil parameter is shown in Appendix 1.

Soil moisture was similar between the *Isoglossa* treatments ($F_{1,\ 34}$ = 0.09, P = 0.762) but varied with time ($F_{3,\ 108}$ = 35.94, $P < 0.001$). Soil moisture followed a seasonal pattern; it was similar between treatments in June but attained slightly higher levels in the *Isoglossa*-removed than in the *Isoglossa*-intact sub-plots in the dry season months of August and October (Fig. 2). In April, soil moisture levels were insignificantly lower in the *Isoglossa*-removed than in the *Isoglossa*-intact treatment (Fig. 2).

Mean monthly temperatures did not vary between the *Isoglossa* treatments at midday ($F_{1,\ 146} = 0.630$, $P = 0.429$, Fig. 3).

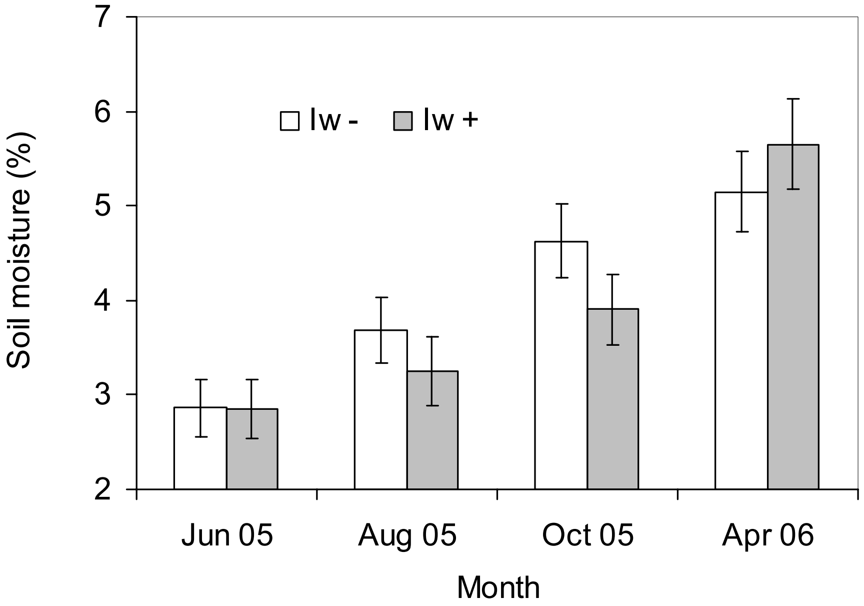

Fig. 2. Mean (± s.e.) gravimetric soil moisture in *Isoglossa*-removed and *Isoglossa*-intact subplots at Cape Vidal during 2005 and 2006.

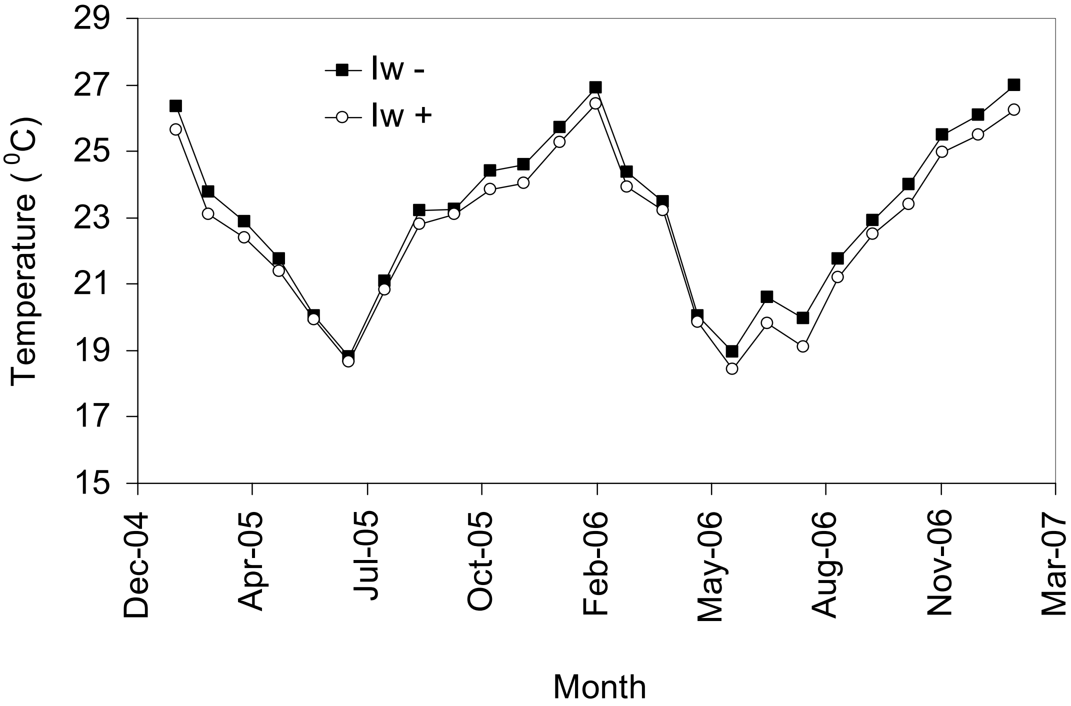

Fig. 3. Midday temperature profiles in the *Isoglossa*-intact and *Isoglossa*-removed treatments at Cape Vidal from February 2005 to February 2007.

Tree seedling density and species composition

Tree seedling density varied significantly between the *Isoglossa* and herbivory treatments among plots and over time (Table 1). The interaction of *Isoglossa* and herbivory was, however, not significant among plots and over time (Table 1). When data from Plot 18 were included in the analysis, tree seedling density in both the *Isoglossa* and herbivory treatments was significantly different among plots but not over time (*Isoglossa*: $F_{7,\ 504}$ = 3.39, P = 0.061; herbivory: $F_{7,\ 504}$ = 0.97, P = 0.345). The *Isoglossa* × herbivory interation was not significant as before. The effect of the *Isoglossa* understorey on tree seedling density was greater than that of herbivory, as shown by a greater difference between the *Isoglossa* treatments without herbivory than between the herbivory treatments without *Isoglossa* (Fig. 4a). The strong effect of herb cover became evident as early as 2002 (two years after *Isoglossa* dieback), while herbivore effects only manifested from 2005 (Fig. 4a). Herbivore effects were greater in the gaps than under the understorey. During the eight years of the study, tree seedling densities remained almost constant in the control treatment (unfenced (herbivory) with *Isoglossa*-intact treatment) while the other three treatments had greater increases in seedling density with time (Fig. 4a). Inclusion of data from Plot 18 changed the trends in seedling density for the unfenced (herbivory) with herb-removed treatment between 2001 and 2007 (Fig. 4b). The sub-plot that received this treatment was in the seed-shadow of a *S. zeyheri* tree that masted in 2007.

Species richness varied significantly between the *Isoglossa* cover and herbivory treatments among plots and over time (Table 2). The interaction of the *Isoglossa* and herbivory treatments over time was significant ($F_{7, 504} = 3.67$, $P = 0.006$). The effect of *Isoglossa* cover on tree seedling species richness was similar to that on density: evident by 2002, while herbivore effects only became strongly evident in 2006 (Fig. 4c). Tree species richness hardly increased between 2001 and 2008 in the unfenced, *Isoglossa*-intact treatment but almost doubled or more than doubled in the other treatments during the same period (Fig. 4c).

Many tree species were represented at the seedling stage in both *I. woodii* thickets and gaps but only a few tree species (e.g., *Diospyros natalensis, Ochna natalitia, Teclea gerrardii*) were common (Fig. 5). Several tree species were either rare (*Dovyalis longispina, Eugenia woodii, Strychnos gerrardii*) or were absent from understorey thickets (e.g., *Canthium inerme, Mystroxylon aethiopicum, Peddiea africana*). Species in the *I. woodii* thickets were therefore a subset of those that occur in understorey gaps. Some species that were rare at the seedling stage are nevertheless relatively common trees in coastal dune forest (e.g., *D. longispina, S. gerrardii, Ziziphus mucronata,* Venter 1976).

The 2004 paired plots survey showed that the density of tree seedlings was higher in understorey gaps compared to areas with *I. woodi* cover ($t = 2.76$, df = 58, $P = 0.008$). Tree species richness also increased between the understorey habitats ($t = 3.2$, df = 58, $P = 0.002$). Using data from the permanent plots survey in 2004, tree seedling density increased from understorey cover to gaps, and was greater than densities from the paired plots (Table 3). Tree seedling species richness followed the same trend but was similar between the two surveys (Table 3).

Table 1. Repeated measures analysis of variance for seedling density in the *Isoglossa* and herbivory treatments.

Source of Variation	df	MS	*F*	*P*
Isoglossa	1	8084.39	46.57	<0.001
Herbivory	1	3878.14	22.34	<0.001
Isoglossa × Herbivory	1	641.46	3.7	0.060
Residual	54	173.59	5.84	
Time	7	1133.44	38.15	<0.001
Time × *Isoglossa*	7	255.77	8.61	<0.001
Time × Herbivory	7	161.82	5.45	<0.001
Time × *Isoglossa* × Herbivory	7	13.87	0.47	0.763
Residual	500	29.71		
Total	603			

Data for Plot 18 in 2008 were removed from the analysis

Using an information theoretic approach, none of the soil fertility variables were important in explaining tree seedling density or seedling species richness and did not significantly improve on the fit of the null model. The best-fit model to the seedling density data was one that included the effect of only Ca (Table 4). Although total soil nitrogen had an AIC value slightly lower than that of the null model (10.95 vs 10.96), its influence was minor. In contrast to seedling density, which was not correlated with total soil nitrogen, species richness was influenced by total soil nitrogen (Fig. 6).

Table 2. Repeated measures analysis of variance for species richness in the *Isoglossa* and herbivory treatments.

Source of Variation	df	MS	*F*	*P*
Isoglossa	1	720.796	117.13	<0.001
Herbivory	1	142.939	23.23	<0.001
Isoglossa × Herbivory	1	20.192	3.28	0.076
Residual	54	6.154	8.54	
Time	7	72.708	100.93	<0.001
Time × Isoglossa	7	22.442	31.15	<0.001
Time × Herbivory	7	8.007	11.11	<0.001
Time × Isoglossa × Herbivory	7	2.641	3.67	0.006
Residual	504	0.720		
Total	607	2508.827		

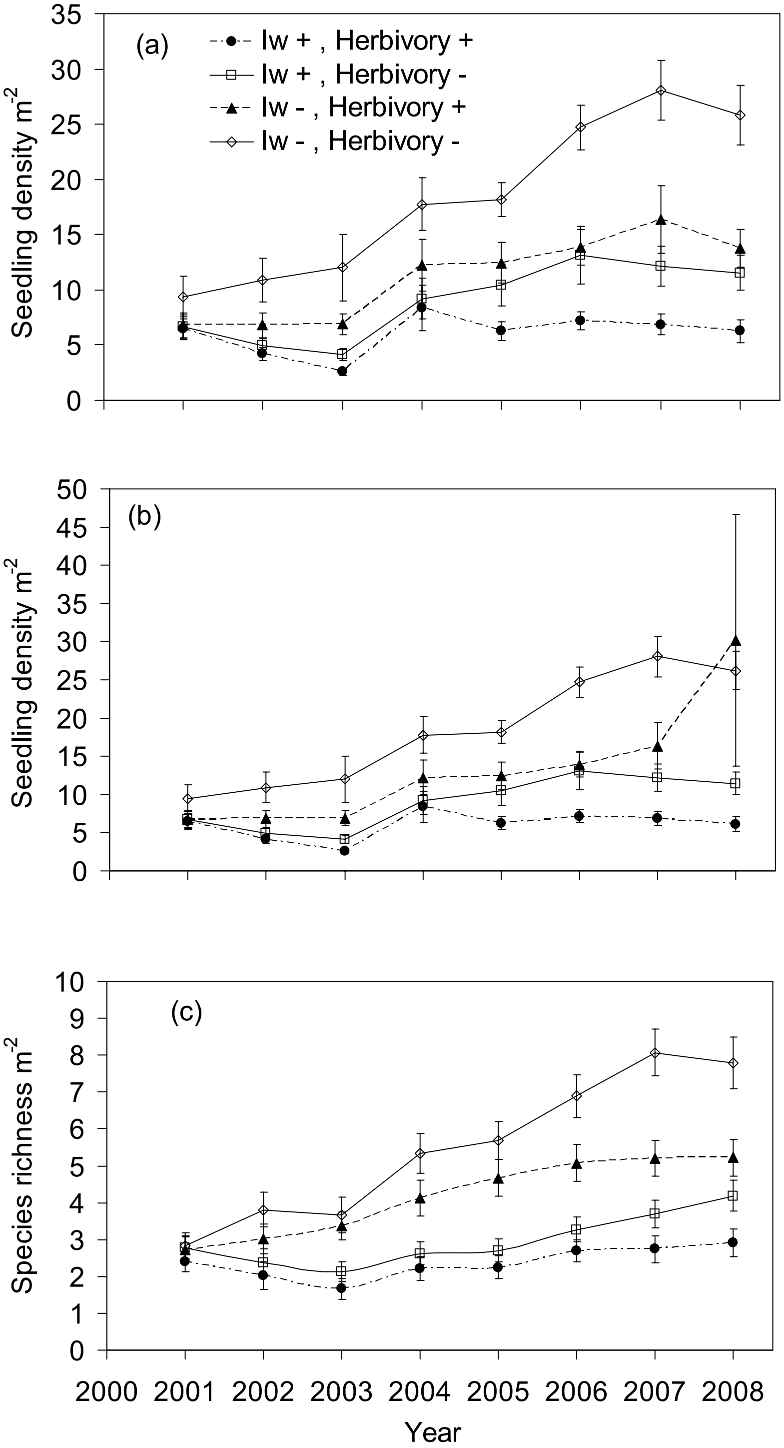

Fig. 4. Mean (± s.e.) seedling density (a, b), and species richness (c) under *Isoglossa* (*Isoglossa*, No *Isoglossa*) and herbivore (Herbivory, No Herbivory) treatments. In (a), seedling density excludes Plot 18 data, which is included in (b).

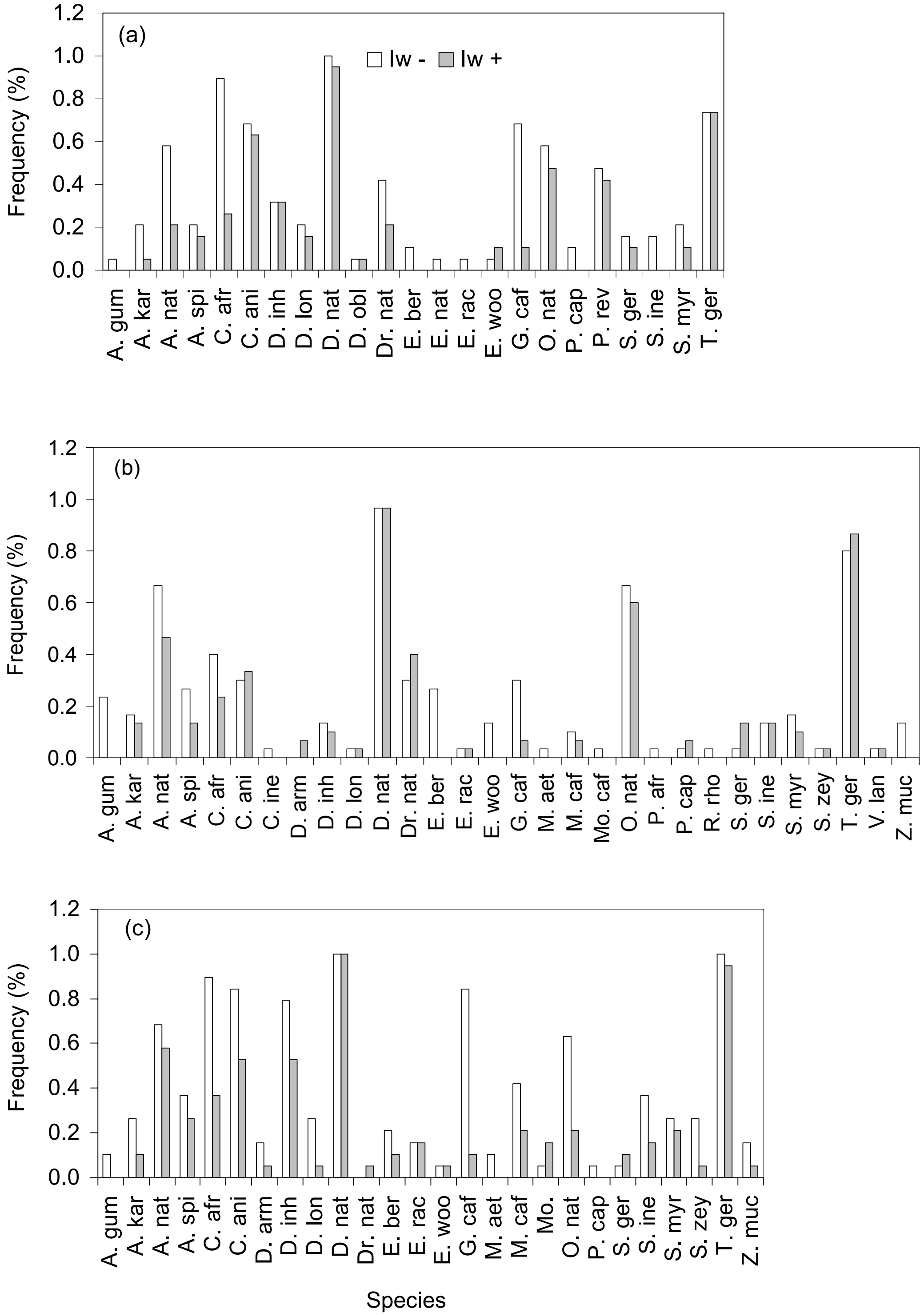

Fig. 5. Occurrence (frequency, %) of seedlings of tree species in *Isoglossa woodii*-removed (Iw -) or intact plots (Iw +) (a, c) and in paired plots of *I. woodii* gaps and thicket (b) at Cape Vidal. Measurements were made in 2003, 2004 and 2006 for (a), (b)

and (c) respectively. Species names are A. gum (*Adenia gummifera*), A. kar (*Acacia karroo*), A. nat (*Allophylus natalensis*), A. spi (*Adenopodia spicata*), C. afri (*Celtis africana*), C. ani (*Clausena anisata*), C. ine (*Canthium inerme*), D. arm (*Dalbergia armata*), D. inh (*Diospyros inhacaensis*), D. lon (*Dovyalis longispina*), D. nat (*Diospros natalensis*), D. obl (*Deinbollia oblongifolia*), Dr. nat (*Drypetes natalensis*), E. ber (*Erythroccoca berberidea*), E. nat (*Euclea natalensis*), E. rac (*E. racemosa*), E. woo (*Eugenia woodii*), G. caf (*Grewia caffra*), M.aet (*Mystroxylon aethiopicum*), M. caf (*Mimusops caffra*), Mo. caf (*Monanthotaxis caffra*), O. nat (*Ochna natalitia*), P. afr (*Peddiea africana*), P. cap (*Pavetta capensis*), P. rev (*P. revoluta*), R. rho (*Rhoicissus rhomboidea*), S. ger (*Strychnos gerrardii*), S. ine (*Sideroxylon inerme*), S. myr (*Scutia myrtina*), S. zey (*Scolopia zeyheri*), T. ger (*Teclea gerrardii*), V. lan (*Vepris lanceolata*), and Z. muc (*Ziziphus mucronata*). Nomenclature follows Coates Palgrave (2002) except for *Isoglossa woodii* when it follows Arnold and de Wet (1993). Note that (a), (b), and (c) are not drawn to same scale.

Table 3. Density and species richness of tree seedlings from the permanent and paired plot surveys at Cape Vidal in 2004.

Variable	Permanent Plots		Paired Plots	
	Iw +	*Iw* -	*Iw* +	*Iw* -
Seedling density (m^{-2})	8.37 (2.05)	12.23 (2.31)	3.57 (0.31)	4.82 (0.33)
Species richness (m^{-2})	2.21 (0.31)	4.13 (0.50)	1.96 (0.13)	2.59 (0.14)

Notes

Iw + and *Iw* – refer to presence and absence of an *Isoglossa woodii* understorey. Values reported are means with the standard error of the mean in parentheses.

Table 4. Summary of regression analysis for seedling density in the fenced, *Isoglossa*-removed sub-plots in 2006. ΔAIC_c is the difference between a model's AIC_c value and the minimum AIC_c value among all the candidate models set; w_i is the Akaike weight, which is the probability that model *i* is the best in the model set; explained deviance is the reduction in residual deviance as a proportion of null deviance. Models are ranked in descending order of w_i, and only models with an $\Delta AIC_c < 10$ are shown.

Model	AIC_c	Δ AIC_c	w_i	Explained deviance (%)
Ca	56.7	0.1	0.48	0.8
Total nitrogen + Ca	59.1	2.6	0.14	6.0
Ca + Zn	59.2	2.6	0.14	0.7
Total nitrogen	61.2	4.6	0.05	0.4
Total nitrogen + pH	62.4	5.9	0.03	2.0
Total nitrogen + water content	63.7	7.1	0.01	0.6

Tree seedling survivorship

Seedlings of the seven important tree species showed increasing survivorship between the third and sixth years of the experiment. *Allophylus natalensis* was absent from all plots in 2002 but was represented in all the *Isoglossa*-herbivory treatments in 2005 with > 60 % of seedlings present in 2005 surviving to 2006 (Fig. 7). In both time periods, no *Celtis africana* seedling survived in the *I. woodii* present sub-plots, but in the other sub-plots > 50 % of seedlings present in 2005 survived to 2006 (Fig. 7). For the rest of the important tree species (e.g., *Clausena anisata*, *Diospyros natalensis, Teclea gerrardii*), survival exceeded 40 % in 2006 and for each of the species some seedlings survived in all four *Isoglossa*-herbivory treatments (Fig. 7). The influence of the *I. woodii* understorey on seedling survival was greater than that of herbivory for all species except *T. gerrardii*, where herb and herbivore effects were of similar magnitude in both years.

None of the seedlings of four less important tree species present in 2002 survived to 2003 (Fig. 8). For *A. dimidiata*, survival was nil in both years. As reported for the important species above, the *I. woodii* understorey had a greater effect on seedling survival than herbivore effects, which were evident in 2006 but not in 2002 (Fig. 8). However, too few seedlings survived for comparison of the relative effects of *I. woodii* and herbivory to be made for *Eugenia woodii*, *Grewia caffra* and *Mimusops caffra* in 2006 (Fig. 8).

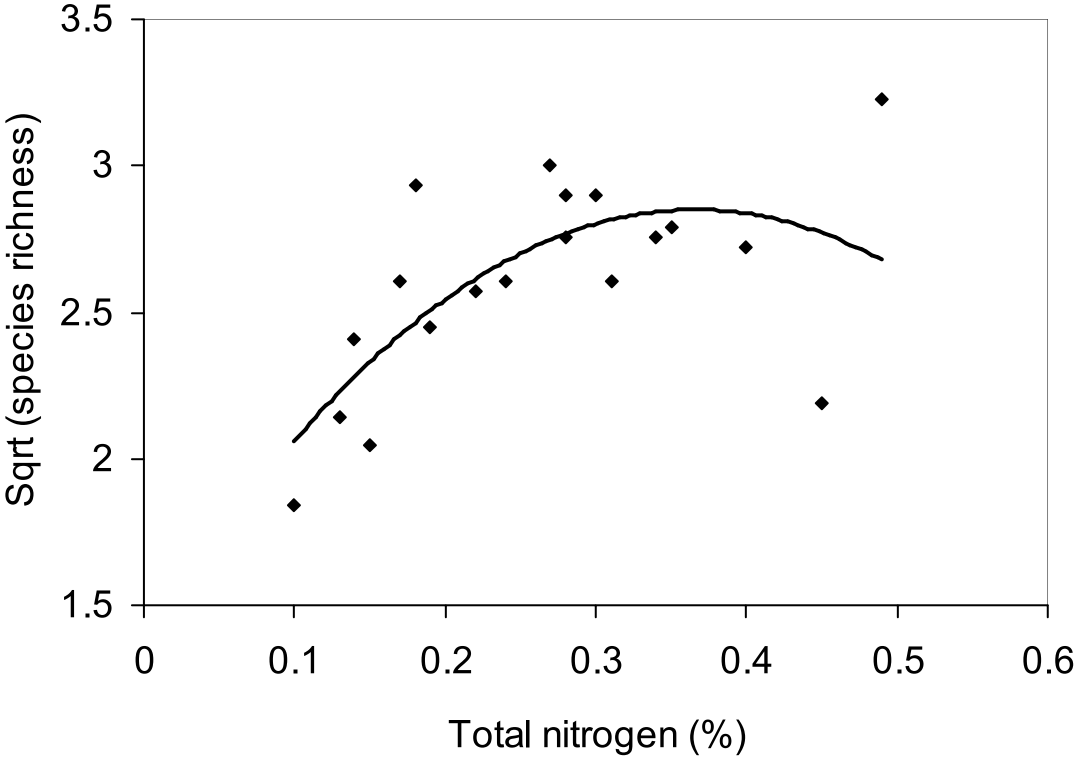

Fig. 6. Relationship between species richness and total nitrogen in the fenced, *Isoglossa*-removed sub-plots at Cape Vidal during 2006 ($y = 1.355+8.177x-11.18\,x^2$, $F_{2,\,16} = 6.93$, $P = 0.007$, $R^2 = 0.397$).

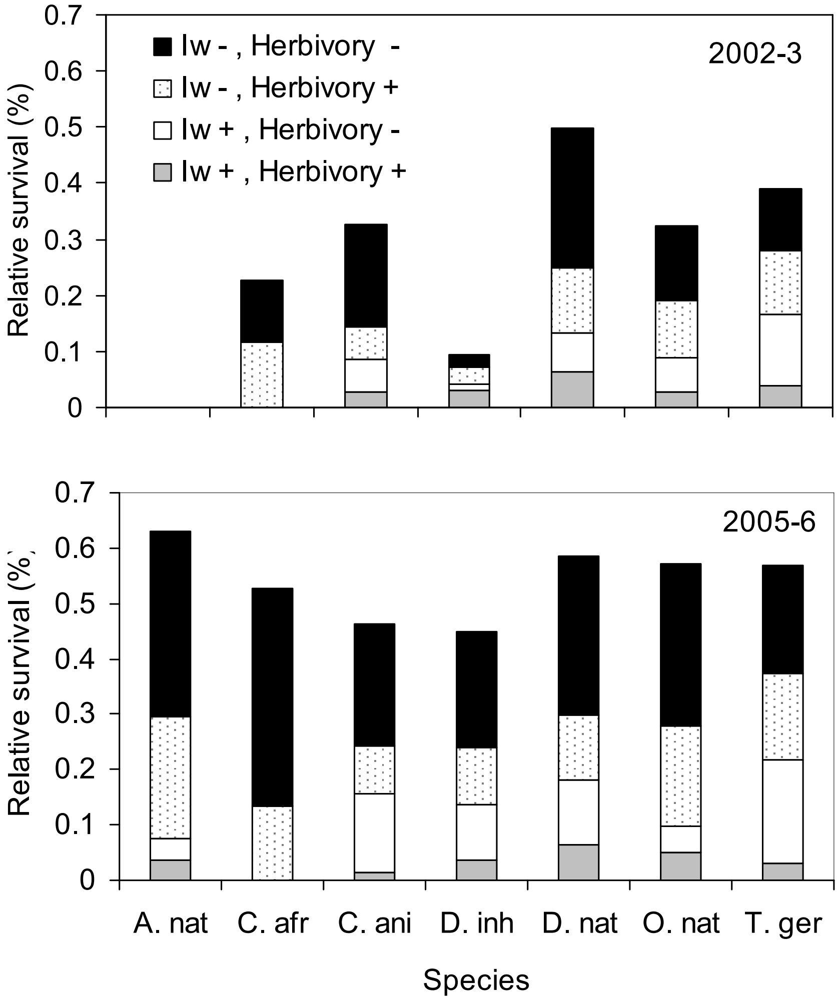

Fig. 7. Relative survival (%) of tree seedlings of main (important) species during 2003 and 2006 in the *Isoglossa woodii*-herbivory treatments. Species' names are as in Fig. 5.

DISCUSSION

We found strong effects of the *I. woodii* understorey in reducing tree seedling density, species richness and survival. Herbivory had similar suppressive effects on seedling recruitment, but the magnitude of seedling suppression by *I. woodii* was greater than that by herbivory. These findings are commensurate with the findings of Reed and Clokie (2000) of greater suppression of tree regeneration by *Mimulopsis alpina* (also an Acanthaceous herb) than by grazing in an Afromontane forest in the Mount Elgon

National Park in Uganda. In addition, Paul et al. (2004) reported lower seedling densities in *Acanthus pubescens*-dominated areas than in areas of the forest without *A. pubescens* cover. Our findings contrast with those of Lawes and Chapman (2006), who reported greater herbivore than herb effects on seedling abundance in disturbed Afrotropical forest in Uganda. In their study, Lawes and Chapman (2006) found that although tree seedlings were more abundant in areas cleared of an *Acanthus pubescens* herb layer, elephants indirectly affected seedlings through damaging and trampling the *A. pubescens* layer. It is noteworthy that elephant effects on tree seedlings were greater beneath intact understorey. Ickes et al. (2001, 2003) have also shown that a native wild pig, *Sus scrofa*, strongly reduces tree recruitment by removal of nearby saplings of a certain size range for nest building in a lowland rain forest in Malaysia.

Our finding of an effect of *I. woodii* cover on seedling density only two years after the dieback suggests that *I. woodii* exerts its influence on seedlings even when *I. woodii* is relatively young and small. During this time, the suppression of tree seedlings by seedlings of *I. woodii* may occur through competition for belowground resources. As *I. woodii* grows and becomes taller than tree seedlings, it influences the tree seedlings through competition (Chapter 6) and through modifying the light environment in the understorey (Chapter 7). The availability of light is considered a primary factor limiting seedling establishment in forests (Chazdon et al. 1996, Kitajima 1996, Agyeman et al. 1999). This has been confirmed in forest understories dominated by ferns, palms, rhododendrons and bamboos (Denslow et al. 1991, Horsley 1993, Clinton and Vose 1996, González et al. 2002, George and Bazzaz 2003, Farris-Lopez et al. 2004).

Although the distribution of *I. woodii* in the forest has been classified into understorey gaps and thickets, patterns of tree seed predation are not related to the presence or absence of *I. woodii* (Tsvuura et al. 2007). However, there is evidence that mortality of *T. gerrardii* seedlings is higher in the *I. woodii* understorey than in *I. woodii* gaps (Tsvuura et al. 2007). Consistent with this finding on *T. gerrardii*, our results showed that most of the species had greater survival in the gaps than under *I. woodii* cover. Some tree species (e.g., *A. dimidiata*, *A. karroo*, *C. africana*, *D. longispina*, *G. caffra*, *M. caffra*) did not survive in the understorey with herbivory. The differential effects of understorey vegetation on survival and subsequent establishment of tree species has led to the notion of an ecological filter that shapes the structure and species composition in tropical and neotropical forests (Denslow et al. 19991, George and Bazzaz 1999a, b, 2003). The ecological filter model is consistent with the findings of Griffiths et al. (2007), who reported that the species pool under *I. woodii* was a subset of that in understorey gaps. The regeneration of tree species whose seedlings are excluded by *I. woodii* cover is therefore made possible in *I. woodii* gaps. The relatively high occurrence of understorey gaps in coastal dune forest (estimated at 5-35 %, Griffiths et al. 2007) suggests that the opportunities for regeneration of these species are high. For example, in spite of being rare at the seedling stage in the understorey, *A. karroo*, *C. africana, D. longispina* and *M. caffra* are common trees in coastal dune forest (Venter 1976, Nzunda et al. 2007a, b), which indicates that seedling recruitment may be greater than is shown by seedling abundance in the understorey (see below).

Regeneration of tree species in *I. woodii* gaps may be akin to the canopy gap-phase regeneration (*sensu* Brokaw 1985) in the neotropics but there are major differences between these gaps in terms of their structure, longevity and frequency of occurrence. First, the amount of light in large canopy gaps may increase to approach photosynthetically-saturating levels of photosynthetically active radiation (PAR) (Chazdon 1986, Agyeman et al. 1999) while levels in *I. woodii* gaps remain below 10 % of PAR (Griffiths et al. 2007). Second, closure of canopy gaps depends on lateral ingrowth of branches of nearby trees or from advanced regeneration, while closure of *I. woodii* gaps depends on sufficient seed being dispersed into the gaps during the reproductive phase of the herb and successful establishment of its seedlings. As *I. woodii* seed is largely gravity-dispersed (Chapter 5), the gap phase may be longer than the life cycle of the species and also longer than the 4-year gap phase in many canopy gaps (Fraver et al. 1998). Finally, understorey gaps occupy a larger fraction of the forest (see above) compared to the 4-6 % in neotropical forests (Hartshorn 1978, Sanford et al. 1986, Fraver et al. 1998) and the 7.8 ± 0.8 % in coastal scarp forest (Obiri and Lawes 2004). The significance of these differences is two-fold: (i) tree recruitment in understorey gaps may occur over a longer time period than in canopy gaps; and (ii) the larger proportion of the forest occupied by understorey gaps may increase the potential for regeneration of tree species that are excluded at other sites by understorey vegetation.

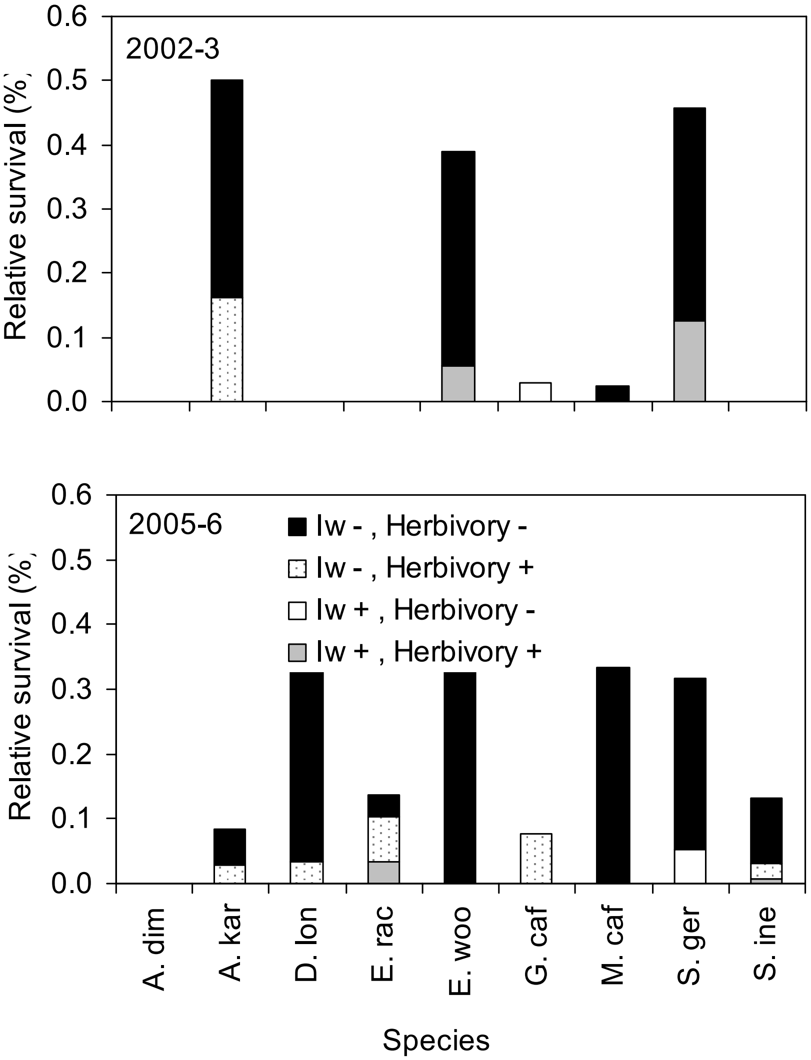

Fig. 8. Relative survival (%) of seedlings of minor (not important) species in the permanent plots during 2003 and 2006. Species names are as in Fig. 5; A. dim is *Apodytes dimidiata*. There were 0-31 surviving seedlings per species in 2003 (out of 0-89) and 0-15 seedlings in 2006 (out of 3-130).

Post-reproductive mortality of *I. woodii* results in the whole forest floor becoming a relatively short-lived understorey gap. As *I. woodii* competes with tree seedlings resulting in suppression of their growth (Chapter 6), seedlings of tree species already established in the forest will be released from competition by the dieback of *I. woodii*, while the increased light conditions may increase seedling growth and survival (Popma and Bongers 1988, Uhl et al. 1988) resulting in greater diversity (Schnitzer and Carson 2001, see also Chapter 7). In contrast, Uhl et al. (1988) and Hubbell et al. (1999) have argued that the greater tree seedling diversity in gap sites compared to non-gap sites is an artifact of higher seedling densities in the gaps. However, our results of tree seedling densities from the paired and permanent plot surveys show that in spite of the differences in density between these surveys, the species richness of tree seedlings in gap sites is still greater than that of non-gap sites. This suggests that the species richness of tree seedlings in understorey gaps is not explained by seedling density alone. The periodic dieback of *I. woodii* thus results in understorey conditions that favour the regeneration of more tree species than is realised under *I. woodii* as indicated (Chapter 7 and Griffiths et al. 2007).

The increased levels of light resulting from monocarpic senescence of *I. woodii* may be insufficient to promote the establishment of light demanding tree species. In fact, our ecological filtering model suggests that the *I. woodii* habitat has selected against the light demanding functional group of tree species (see Chapter 7 and Griffiths et al. 2007), which has been largely lost over time and is represented in the forest by only a few pioneer species such as *C. africana*. An important consequence of the habitat filter is the abundance of species that belong to the shade-tolerant guild. This guild comprises species that are tolerant of deep shade (e.g., *C. anisata*, *Diospyros natalensis*, *D. inhacaensis*, *Drypetes natalensis*, *Ochna natalensis* and *T. gerrardii*) (see also Chapter 7), as well as species that are moderately shade-tolerant (see above). *Isoglossa woodii* thus acts as a filter that selects for convergence in traits needed to cope with establishment conditions imposed by its dominance in the forest.

The greater effects of herbivory on seedling density in gaps than under the understorey may dampen the density differences between these habitats. However, the effect of the understorey was greater than that of herbivory, resulting in the maintenance of tree seedling density differences between the understorey habitats. Although herbivores affected the species composition of the tree seedling community in gap and understorey habitats, differences in those effects between the habitats were minor. This indicates that herbivory may reduce tree seedling density but not species composition, which is in contrast to Liang and Seagle (2002), who reported decreased tree seedling diversity due to deer browsing in a riparian forest in Maryland. In addition, Veblen et al. (1989) found that browsing by exotic deer in a Patagonian rainforest has nearly eliminated *Aristotelia chilensis*, an understorey dominant in areas unaffected by deer.

We found no effects of understorey vegetation and herbivory on soil fertility and soil moisture. The similar levels of nutrients we found between the *Isoglossa* habitats and between the herbivore treatments are consistent with the findings of earlier studies on effects of the *I. woodii* understorey on soil properties (Boyes 2007, Griffiths et al. 2007). In the Boyes (2007) study, soil nutrients (e.g., total N, P) were compared between the dune forest and nearby stands of *Acacia karroo*, both with an *I. woodii* understorey. She found similar levels of these nutrients in the dune forest and in the *A. karroo* stands. Griffiths et al. (2007) compared soil nutrient levels in *Isoglossa* thickets and in areas without an *Isoglossa* cover and found that nutrient levels were not significantly different between these habitats. Herbivore exclusion and the *Isoglossa* understorey therefore have neutral effects on soil fertility. Other studies of forest understories have shown no effect of understorey plants on soil fertility (Horsley 1993, Carlton and Bazzaz 1998, George and Bazzaz 1999a). For example, Horsley (1993) measured total nitrogen and phosphorus in areas under fern cover and areas without fern cover and reported similar levels of these nutrients between the fern habitats. George and Bazzaz (1999a) also reported similar levels of soil organic matter in fern and fern-free areas. In contrast, Nilsen et al. (2001) found that a *Rhododendron maximum* understorey vegetation reduced availability of several nutrients in mixed-hardwood forests of southeastern United States.

The absence of an effect of *I. woodii* on soil water content is not surprising considering the species' shallow adventitious rooting system. Studies carried out on the effects of an understorey fern stratum have shown that fern cover does not affect soil moisture (Horsley 1993, Carlton and Bazzaz 1998, George and Bazzaz 2003). In contrast, Clinton and Vose (1996) and Nilsen et al. (2001) have shown that the *Rhododendron maximum* understorey in mixed-hardwood forests in the southern Appalachian Mountains reduced the availability of soil water by at least 20%. Differences in types of understorey cover may therefore explain the soil water relations reported in this and other studies.

Although there was no relationship between tree seedling density and soil water content, the low availability of soil moisture in these forests (Venter 1976) may have severe impacts on tree seedlings; more so during drought and winter dry spells when severe wilting of *I. woodii* plants is common (Z. Tsvuura, personal observations). There is evidence of density-dependent size asymmetric competition between *I. woodii* and tree seedlings (Chapter 6), which results in suppression of woody seedlings. For tree seedlings in the understorey during the vegetative phase of *I. woodii*, establishment may therefore depend on their ability to survive in a microenvironment characterised by deep shade, severe competition for belowground resources, periodic moisture deficits and herbivory. In constrast, tree seedlings in understorey gaps are exposed to relatively higher light, less severe competition and greater herbivory.

The combined effects of herbivory and understorey vegetation therefore curtails tree seedling establishment, thereby influencing the structure and species composition of the forest. The presence of gaps in understorey cover provides opportunities for regeneration of tree species that are sensitive to conditions associated with *I. woodii* cover. Monocarpic senescence of the dominant understorey species creates conducive gap conditions for tree seedling establishment but these conditions are short-lived due to the high density and rapid growth of *I. woodii*. The gap conditions created by the dieback of *I. woodii* may thus benefit advanced regeneration rather than establishment of new tree seedlings, and thus impose selection pressure on tree species capable of establishing beneath the understorey (i.e., selecting for shade-tolerance). Contrasting microenvironments between gap and understorey sites therefore provide the suite of conditions required for the maintenance of tree species diversity in coastal dune forests.

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APPENDICES

Appendix 1. Mean values of soil parameters in soil samples collected from *Isoglossa*-intact and *Isoglossa*-removed plots at Cape Vidal. The standard error of the mean is shown in parenthesis.

Parameter	*Isoglossa*-removed	*Isoglossa*-intact
Bulk density (g/cm^3)	1.3 (0.01)	1.3 (0.01)
P (mg/kg)	22.1 (1.8)	23.6 (1.5)
K (mg/kg)	83.2 (3.5)	86.7 (3.1)
Ca (mg/kg)	2468.6 (100.5)	2472.8 (91.2)
Mg (mg/kg)	212.6 (17.0)	225.1 (12.9)
Exchangeable acidity (cmol$_c$/L)	0.05 (0.01)	0.06 (0.01)
pH (KCl)	7.2 (0.1)	7.3 (0.1)
Zn* (mg/L)	1.6 (0.1)	1.63 (0.1)
Mn (mg/kg)	2.8 (0.1)	2.82 (0.1)
Clay (%)	10.8 (0.5)	10.8 (0.5)
Cu* (mg/L)	0.8 (0.04)	0.7 (0.03)
Total Carbon (%)	3.0 (0.2)	2.7 (0.1)
Total Nitrogen (%)	0.3 (0.02)	0.2 (0.01)
Total Cations (cmol$_c$/L)	14.33 (0.6)	14.47 (0.5)

* Data were square-root transformed before analysis.

CHAPTER 9

SUMMARY AND CONCLUSIONS

This thesis examined the influence of a monodominant understorey herb, *Isoglossa woodii*, on tree seedling dynamics and species composition in Indian Ocean coastal dune forest in KwaZulu-Natal Province, South Africa. The role of understorey vegetation as an ecological filter that shapes forest tree composition and structure has received increasing attention in the recent past (Denslow et al. 1991, George and Bazzaz 2003, Nilsson and Wardle 2005, Gaxiola et al. 2008). Understorey species have been shown to be important ecological filters, suppressing tree regeneration and thereby affecting the forest community in several ways. For example, suppressive effects on tree regeneration may change the species composition of the forest leading to the prevalence of species with particular combinations of traits, as shown in this study.

The life history of *I. woodii* comprises a relatively long vegetative stage that is followed by synchronized reproductive and mortality events, which may have important implications for forest regeneration, and ultimately for forest structure and composition. The species occurs in dense stands in the forest understorey but is locally absent from some sites thereby forming understorey gaps. The long vegetative stage of the species results in long periods of deep shade on the forest floor that consequently selects for shade-tolerant species over light-demanding species. The monocarpic cycle provides a recruitment window for seedling establishment at dieback of the herb, but because of its short-lived nature, it enhances establishment of advanced regeneration. Therefore, in addition to the selective filter that *I. woodii* imposes on forest dynamics, the spatial distribution of the herb species and its evolutionary and reproductive biology, presents a unique opportunity for understanding how an understorey plant may affect the community dynamics and species interaction of the overstorey in subtropical forest.

The main aim of this study was to clarify the role of *I. woodii* in driving canopy tree species establishment and dynamics. A secondary aim was to determine the reproductive biology of *I. woodii,* and the selective forces that may have shaped it. The objectives of this study were:

(i) to investigate the ecological and environmental correlates of the distribution of *I. woodii* in dune forest;

(ii) to investigate the roles of outcrossing and predator satiation as evolutionary advantages of synchronous monocarpy in *I. woodii*; and

(iii) to determine the ecological effects of the extensive cover and putative recruitment window caused by *I. woodii* on coastal dune forest tree seedling dynamics and diversity.

Influence of gaps in the herb understorey on woody seedling regeneration

To understand how the *Isoglossa woodii* understorey influences seedling regeneration, the extent of understorey cover as well as the availability of light in understorey habitats (areas with and without *I. woodii* cover) were measured. *Isoglossa woodii* covered 65-95 % of the understorey, with gaps in the species' cover making up the remainder. Light levels were < 1% of photosynthetically active radiation (PAR) beneath *I. woodii* and 8 % of PAR in the gaps. Similarly low light levels have been reported in the understoreys of temperate and tropical forests (Denslow et al. 1991, Horsley 1993, George and Bazzaz 1999, 2003, Farris-Lopez et al. 2004, Gaxiola et al. 2008). The herb thus clearly shades out the forest floor and provides a challenging light environment for establishing woody seedlings. This is one way in which the herb acts as an ecological filter.

To reveal the association between understorey cover and tree seedling recruitment, the density and species composition of the tree seedling community in the understorey habitats was investigated. *Isoglossa woodii* had a strong suppressive effect on seedling abundance and species composition. Seedling density and species composition were greater in the gaps than in the understorey, with more species confined to the gaps than in the understorey, which suggests that *I. woodii* creates conditions that limit tree regeneration, a finding that is consistent with several studies of the effect of understorey vegetation (Denslow et al. 1991, Farris-Lopez et al. 2004, LaFrankie and

Saw 2005, Nilsson and Wardle 2005). The relationship between the distribution of *I. woodii* and the proximity to the ocean, topography, soil fertility and community structure of the tree canopy were also investigated (Chapter 2). The density of *I. woodii* increased with distance from the ocean and was negatively correlated with canopy tree cover, while its height and density varied with topographic location. *Isoglossa woodii* was tallest in the dune slacks and shortest and most dense on the dune crests (Chapter 2). The light environment probably accounts for this, for dune slacks are more shaded than dune crests. None of the soil fertility parameters were associated with the distribution of *I. woodii*, which indicates that the species does not influence soil characteristics and neither is it limited in its distribution by the variation in soil fertility at this site (Chapters 2 and 8).

Seed predation and seedling survival

The possibility that *I. woodii* influences tree seedling regeneration in coastal dune forest by increasing fruit predation and seedling herbivory was tested. Patterns of fruit predation were not related to the presence of understorey cover for the species tested, which shows that factors other than fruit predation limits tree recruitment in dune forests (see below). Studies elsewhere have shown that understorey vegetation may influence seed predation by providing habitat to predators (e.g., Wada 1993, Holl 2002) or by providing habitat to predators of seed predators (Schupp and Frost 1989). However, seedling survival for one of two tree species tested showed an understorey effect, which indicates that post emergence processes such as herbivory are important in tree regeneration in these coastal forests (Sork 1987, Fuchs et al. 2000, Ickes et al. 2003, Boyes 2007).

Reproductive biology of *I. woodii*

A species that flowers synchronously among individual plants may benefit from outcrossing (Augspurger 1980, Stephenson 1982, Burd 1994). However, this may be more effective if the species is pollinated by wind because animal pollinators may be satiated (Kelly and Sork 2002). The breeding system of *I. woodii* was investigated during a synchronous flowering event. Employing both self- and cross-fertilization, the species has a mixed mating strategy (Griffiths et al. 2009), which is common among seed plants

(Goodwille et al. 2005, Johnston et al. 2009). Although selfing in species that flower synchronously may provide reproductive assurance, it reduces pollen export thereby conflicting with outcrossing (Charlesworth and Charlesworth 1987, Lloyd 1992, de Jong et al. 1993). Nevertheless, geitonogamous selfing cannot be avoided since it is a by-product of selection for outcrossing when a large floral display is required to attract pollinators (Lloyd 1992, Harder and Barrett 1995, Goodwille et al. 2005, Brunet and Sweet 2006). Regardless, *I. woodii* has a number of traits that promote outcrossing (e.g., high inter-plant flowering synchrony, protandry, acropetally, high pollen-ovule ratio). When pollinator visits to inflorescences commence on the lower, oldest and predominantly female phase flowers and then move upwards to the younger and male phase flowers, the lower flowers are likely to receive outcross pollen while the upper flowers export pollen (Lloyd 1992, de Jong et al. 1993). By this mechanism, the species reduces geitonogamy and thus enhances outcrossing.

Supplemental pollination was not effective, indicating that the species is limited by resources other than pollen (Zimmerman and Pyke 1988). Ovaries contain four ovules but most fruits contained only one seed and none more than 2 seed, which raises the question of why the species has four ovules when all four are never fertilized. The few open flowers on an inflorescence at any time results in a longer flowering period, which indicates the conflicting selection pressures on pollinator attraction and saturation in animal pollinated plants (Harder and Barrett 1995, Benítez-Vieyra et al. 2006).

Predator satiation and recruitment in *I. woodii*

Although the benefits of synchronous flowering in long lived plant species have been explained in terms of predator satiation and facilitation of outcrossing (Silvertown 1980, Smith et al. 1990, Kelly 1994, Kelly and Sork 2002), explanations for monocarpic senescence have been elusive (Janzen 1976, Foster 1977, Gadgil and Prasad 1984, Struhsaker 1997, Wilson 1997). To test whether insect consumers of flowers and developing seed may limit seed production, fruit and seed set were measured on inflorescences subjected to insecticide spray and no spray treatments. Seed production was not limited by insect predation. Post-dispersal predation of seed was measured to investigate if satiation of seed predators occurs after a masting event. Seed predation was low, which suggests that seed predators were satiated and lends support to the notion that synchronous reproduction has arisen due to predation pressure (Silvertown 1980, Ashton et al. 1988, Norton and Kelly 1988).

To determine whether establishment of *I. woodii* seedlings is enhanced by mortality of formerly reproductive plants, and why the species is absent in understorey gaps, growth and survival were measured in seedlings transplanted to sites with and without *I. woodii* cover in formerly reproductive and non-reproductive populations, respectively. Seedling establishment was greater among the dying formerly reproductive plants than in the gaps, and was also greater in the gaps than in the understorey of the non-reproductive population. These findings support the hypothesis that ex-reproductive plants die to facilitate seedling establishment (Foster 1977, Gadgil and Prasad 1984, Kitajima and Augspurger 1989), which invokes kin selection as nearby seedlings are closely related (Wilson 1997, Foster et al. 2006) due to gravity seed dispersal. The greater growth of seedlings in gaps than non-gap sites of the mid-phase population sheds no light on why the species is absent from gaps, but supports further the suggestion that post-reproductive mortality of adult plants reduces intraspecific competition between successive generations of *I. woodii* cohorts thereby maintaining local dominance (Simmonds 1980, Struhsaker 1997).

I. woodii - tree seedling competition

Competitive interactions were tested between tree seedlings and *I. woodii*. Woody seedling biomass decreased with *I. woodii* density and light levels. Site fertility had no effect on the *I. woodii*-tree seedling interaction, while the magnitude of biomass suppression by *I. woodii* was similar among the tree species, precluding a hierarchy of competitive ability among the tree species. Relative growth rate also decreased with competitor density, was light-neutral but increased with nutrient levels. The effect of site fertility in influencing the outcome of competitive interactions is equivocal (Grime 1988, 2001, Tilman 1988, 1997, 2007).

A common outcome of competitive interactions among plant species is the reduced performance of subordinate species (Keddy et al. 1997, 2000). In the understorey environment, seedlings are at a size disadvantage when in close proximity to *I. woodii* plants resulting in reduced performance of the seedlings, as this study shows. As these forests experience periodic soil moisture deficits (Venter 1976), the competitive effect of *I. woodii* on seedling establishment and survival will be exacerbated during these periods (Davies et al. 1998), particularly because *I. woodii* is a shallow-rooted species.

In this plant community where tree species have similar competitive abilities in their interaction with *I. woodii*, the herb is unlikely to cause competitive exclusion of tree species. However, less competitive genotypes within a species may be eliminated in the same way that light demanding species are excluded by the habitat filter of *I. woodii* (see below).

Photosynthetic responses and shade tolerance of tree seedlings

The regeneration of tree species in forests is often partitioned into shade-tolerant and -intolerant species based on gap-phase dynamics, which posits that seedlings of shade-tolerant species can establish in the low light conditions of the forest understorey, while shade intolerant species depend on the occurrence of high light levels in canopy gaps (Hartshorn 1978, Brokaw 1985, Schnitzer and Carson 2001, Poorter and Arets 2003). Light response characteristics of seedlings from coastal dune forest showed that there is little variation among species and that species are tightly packed in terms of their light compensation points, apparent quantum yield and rates of dark respiration, all of which are indicative of shade-tolerance. The understorey shade filter may have selected against light-demanding species which have consequently been reduced over time. A result of this is the phenotypic clustering (in shade tolerance) of distantly related plant taxa that is maintained by low intensity and frequency of disturbance.

Ecological effects of *I. woodii* on tree seedlings

To understand the influence of understorey vegetation and herbivory on tree regeneration, understorey vegetation and herbivore effects were manipulated. The species composition, abundance and survivorship of tree seedlings were negatively influenced by the presence of *I. woodii*, but the effects were stronger for some species than others, which indicate the selective influence of the understorey environment on tree species regeneration (Denslow et al. 1991, George and Bazzaz 2003, Montgomery 2004, Wang and Augspurger 2004).

The suppressive effect of understorey vegetation on regeneration was further strengthened by herbivore effects. Herbivore effects also reduced seedling performance (survival, abundance and diversity) but the magnitude of its effect was lower than that of *I. woodii*.

Understorey suppression of regeneration occurs chiefly through light attenuation but this may also be related to competitive interactions for soil moisture and nutrients (Wright 1992). Size-asymmetric competition between tree seedlings and understorey vegetation results in the reduced performance of the seedlings. In addition, many of the tree species are shade-tolerant, which is associated with sensitivity to drought stress because of trait trade-offs between drought and shade tolerance (Huston 1994, Holmgren et al. 1997). In understorey gaps, performance of seedlings is greater because of enhanced light levels and the absence of competition with *I. woodii*. Understorey gap conditions become prevalent during the post-seed dispersal die back of *I. woodii* but are short-lived because its seed germinate immediately and its seedlings grow faster than tree seedlings. The short time that elapsed between die back and the last measurement of seedling responses (4-6 months) may have resulted in the less-pronounced die back effects (Chapter 8). *Isoglossa woodii* thus acts as an ecological filter that selects for convergence in traits (eg., high shade tolerance, high survivorship in shade, similar competitive ability) needed to cope with establishment conditions imposed by its dominance in the forest. However, a few species are absent in areas with and without *I. woodii* cover (*I. woodii* habitats) as seedlings but are relatively common as trees. These species may persist as adults through sprouting rather than through regeneration from seed (Nzunda 2008). The role of this suppressive herb in promoting resprouting and multi-stemming of dune forest trees has not been clearly established.

Conclusions

- Patterns of tree seed predation are not related to presence or absence of *Isoglossa woodii*. Therefore, tree recruitment in coastal dune forest is independent of seed predation but depends on post-emergence processes.
- The mixed breeding system in *I. woodii* has arisen as a result of the need for an inter-plant mass display to attract pollinators. The evolution of geitonogamous

selfing is subsidiary to outcrossing and incidental to intra-plant flowering synchrony.

- In *I. woodii*, predator satiation operates from flowering to post-dispersal stages, so that at any stage of the reproductive process, the impacts of insect predators is low, resulting in higher levels of seed production than would be attained if reproduction were not synchronous.
- Synchronous seedling establishment in *I. woodii* may extend to population level herbivore satiation thereby maintaining the dominance of the species in the understorey.
- Post seeding mortality of *I. woodii* facilitates seedling establishment, which could occur through competitive release (enhanced light levels and access to belowground resources).
- In their interaction with *I. woodii*, seedlings of tree species have equivalent competitive abilities.
- At the seedling stage most of the tree species in coastal dune forest are shade-tolerant. The prevalence of the understorey ecological filter has selected against light-demanding species which have been reduced over time.
- Gaps in understorey cover and the short-lived, community wide understorey gap created by the die back of *I. woodii* may enhance the establishment of tree species.
- Greater herbivore effects in the gaps dampen the magnitude of differences in the seedling community between gap and non-gap sites thereby maintaining the structure and species diversity of the forest.
- Understorey vegetation imposes a suite of traits among tree species of distant relatedness which may lead to phenotypic convergence. The suite of traits includes but is not limited to similar competitive abilities and light response characteristics.

Directions for future research

Several questions arise from this study. These include the following: What is the proximate cause of flowering in *I. woodii*? Why does an *I. woodii* flower produce only one seed when up to four ovules are available and the species is not pollen-limited? What is the relative fitness of selfed and outcrossed seed? Are rates of predation of *I. woodii* seed that occur at localised scales involving one population realised at regional scales involving more than one population? What is the influence of soil water availability in the interaction between *I. woodii* and tree seedlings? Do the low growth rates of tree seedlings in the forest confirm that the species are shade tolerant (*sensu* Poorter 1999)? Are tree species in coastal dune forest distantly related so that their co-occurrence in the same community can be explained in terms of phenotypic clustering?

The proximate causes of flowering in synchronous species including monocarps are largely unknown (e.g., Read et al. 2006). Only among the Dipterocarpaceae of South East Asia has a drop in minimum nighttime temperature been identified as the immediate cue for flowering (Ashton et al. 1988). *I. woodii* plants collected from Cape Vidal and kept in a green house > 300 km away in Pietermaritzburg for four years flowered at the same time as the parent population in 2007. In addition, cuttings made from young stems and growing tips of these plants and kept in a mist house for root development produced inflorescences and flowered as well, again at the same time as the forest population. It appears that the cue that initiates flowering in this species is independent of environmental factors but may be related to a chemical hormone or an internal calendar (Janzen 1976, Wilson 1997).

Although the ovary of an *I. woodii* flower may contain up to four ovules, most fruits (74-89%) contained only one seed and none had more than 2 seeds (Chapters 4 and 5). The evolutionary advantage to the species of producing more ovules than the realised seed set is an issue worth investigation. It has been suggested that the advantage to the plant of producing nonviable seed is that it increases the amount of time spent by seed predators going through the chaff thereby enhancing survival of viable seed ('the sacrificial sibling hypothesis'; Ghazoul and Satake 2009), provided that predators cannot distinguish between viable and nonviable seed. If selfed seed are of low quality, their prevalence in *I. woodii* may be related to increasing the chances that outcrossed seed will escape predation (see below).

Theoretical models on the evolution of mating systems suggest that mixed mating is evolutionarily unstable and should be selected against (Schemske and Lande 1985), yet empirical evidence shows that mixed mating is relatively common (Holsinger 1991, Goodwille et al. 2005, Griffiths et al. 2009). Since *I. woodii* produced similar quantities of selfed and outcrossed seed and has features that suggest it may be predominantly outcrossed, studies of the relative fitness of selfed versus outcrossed seed are required, as well as examining the relative contributions of self- and cross-fertilization by investigating the paternity of seed from mixed pollinations. This would indicate whether mixed mating in *I. woodii* may have adapative significance or may be transitional and tending towards outcrossing (see Chapter 4).

Although low levels of seed predation in a synchronously seeding population can be considered indicative of predator satiation at local scales (Chapter 5 and Janzen 1976), confirmation of predator satiation requires that small seed crops result in greater predation, and larger seed crops in lower predation (Kelly and Sork 2002). For a monocarpic species, this can be achieved by measuring seed predation in a synchronously seeding and non-seeding population. Therefore, more tentative conclusions regarding predator satiation could have been made if measurement of comparative rates of seed predation in a non-reproductive population had been carried out.

The suppressive effect of understorey vegetation on tree regeneration should be most pronounced during periods of environmental adversity such as drought. Periodic moisture deficits in dune forest may have a strong influence on *I. woodii*-tree seedling competition because the distribution of their roots overlap for soil resources, which suggests that soil moisture may be as important as nutrients in forest regeneration (Riegel et al. 1992, Zavala and de la Parra 2005).

Confirmatory studies are required to determine whether growth rates and leaf traits of tree species occurring in coastal dune forest are correlated to the light response characteristics that led to the conclusion that these species are shade-tolerant (Chapter 7). Shade-tolerant species are expected to have low growth rates due to high defense and storage allocation (Kitajima 1994, 1996). In addition, shade-tolerant species have low specific leaf area because they retain their leaves for long periods (Bongers and Popma 1990, Midgley et al. 1995, Kitajima 1996).

Finally, the ecological filter model in coastal dune forests (Chapters 6, 7 and 8) is premised on distantly related species having similar ecological requirements leading to phenotypic clustering. However, a few familial taxa have more than one constituent genus while some genera have more than one constituent species (Chapter 7), which suggest some genetic relatedness among the taxa. A phylogenetic analysis of the tree species in coastal dune forest is required to confirm whether the tree species are distantly related.

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