Rodents and Restionaceae: sex-specific plant-animal interactions in dioecious plants

An investigation into a newly recorded phenomenon in Fynbos and the potential sex-specific effects on plant morphology, nutrition, & physiology in nut-fruited restios.

By

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signed by candidate

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CHAPTER 1

General Introduction & Thesis Structure

General Introduction

Dioecism in plants

The majority of angiosperms are hermaphrodites, meaning that a single plant holds both male and female reproductive structures. More rarely, species exist with separate male and female plants. This condition is known as dioecism and it occurs in close to half of all angiosperm families (Heilbuth 2000).

In dioecious plant species, comparisons between male and female plants have been studied to explain the causes and effects of the separation in male and female reproductive structures. The most common comparisons include growth rate, sex ratios, niche specification, morphology, micro-climate and phenology in conjunction with reproductive effort (Lloyd & Webb 1977; Dawson & Ehleringer 1993). In addition, detailed studies of sex-specific physiology have recently begun to make a contribution to the study of dioecism in plants using tools such as isotopes, nutrient analysis and gas exchange. This has contributed significantly to the study of dioecism because it provides a deeper understanding of the internal plant-processes that are linked to observable male/female plant differences.

The costs of reproduction are often reported to be higher in seed-producing, female plants compared to pollen-producing, male plants (Darwin 1877; Lloyd & Webb 1977; Antos & Allen 1990; Delph 1999; Obeso 2002). Differing reproductive effort (seeds vs. pollen) between sexes can lead to a variety of differences in plant form and functionality. These differences can occur due to sex-specific tradeoffs in the allocation of available resources to various plant processes (Alverez-Cansino *et al* 2010) or due to sex-specific mechanisms and adaptations which compensate for the effects of resource allocation tradeoffs (Dawson & Bliss 1989; Dawson & Ehleringer 1993; Lloyd & Webb 1977). For example, the detectable costs of higher reproductive requirements in female Box Elder, *Acer negundo,* were masked through niche specialisation to wetter habitats and higher photosynthetic capacity which acted as compensatory mechanisms (Dawson & Ehleringer 1993). Differences in physiological performance capabilities are likely to play an important role in facilitating the co-existence of males and females with different reproductive requirements.

Sex-specific plant-animal interactions in dioecious plants

Detailed physiological studies in dioecious plant species do not commonly include the effects of sex-specific plant-animal interactions, presumably because such interactions are rarely recorded. However there have been a number of studies which investigate the effects of herbivory on plant defence, growth and reproductive allocation in dioecious species (eg: Cepeda-Cornejo & Dirzo 2010; Graff *et al* 2013). Sex-specific interactions with animals could lead to significant differences in many aspects of plant form and functionality and are therefore important to consider, if present, in physiological studies of plant dioecism.

In this thesis I documented a previously unrecorded plant-animal interaction in mountain Fynbos which occurs between the dioecious *Cannomois congesta* Mast. (*C. congesta*) (Restionaceae) and *Rhabdomys* (Thomas 1916) (Muridae). In this particular system, the seed predation behaviour of *Rhabdomys* lead to the loss of seeds from female *C. congesta* plants and had noticeable (potentially detrimental) side-effects on female canopy structure. Males were unaffected due to their lack of seeds (see chapter 2 for a detailed description of the sex-specific interaction).

***Study species:* Cannomois congesta**

Cannomois congesta is a species of perennial, grass-like plant belonging to the dioecious Restionaceae family. It has its distribution within the Cape Floristic Region of the Western Cape, South Africa and is common in the South-western mountains and Bredasdorp plains.

The Restionaceae have a unique growth form made up of erect, green, photosynthetic stems (culms) with leaves that have been reduced to leaf sheaths. Inflorescences are made up of many small spikelets which contain tiny, anemophilous flowers. Characteristic of the *Willdenowiea* trine within the Restionaceae, *C. congesta* females produce large, nut-like seeds with an elaiosome attached (Dorrat-Haaksma & Linder 2000; Briggs *et al* 2009). Elaiosomes are fatty, lipid structures attached to certain seeds and are associated with myrmecochory; the dispersal and burial of seeds by ants (Bond & Breytenbach 1985; Lengyel *et al* 2010; Bond & Slingsby 1983). Re-seeders, such as *C. congesta,* are killed by fire and rely completely on seed for the recruitment of the next generation (Linder 1991). Buried seeds are germinated by fire-cues and produce seedlings which face little competition in the post-fire environment (Newton *et al* 2006). Between fire events plants are vulnerable to seed losses, a common threat from rodent seed-predators (Bond & Breytenbach 1985).

Despite their dominance and diversity in many regions, the Restionaceae are relatively understudied compared to other, more charismatic families of the Cape Fynbos (Dorrat-

Haaksma & Linder 2000). In addition to their contribution to the diversity of the Cape Fynbos, the relatively high incidence of dioecism in the Cape flora compared to other temperate floras is due to the large number of Restionaceae species which contribute little under 50% to the incidence of dioecism of the area (Steiner 1988). The sex-specific plant-animal interaction between *C. congesta* and *Rhabdomys* described in chapter 2 might characterise a common phenomenon affecting certain Restionaceae groups (presumably those with attractive, nut-fruited seeds), where *Rhabdomys* (or similar species) is present.

***Study species:* Rhabdomys**

Rhabdomys, also known as the four-striped grass mouse, is a widespread rodent endemic to southern Africa (Bond *et al* 1980; De Graaf 1981). They successfully inhabit a variety of both natural and urbanised habitats and are prone to population explosions (Matthee *et al* 2007). *Rhabdomys* are known to eat a variety of seeds and other foods such as berries and herbs (Bond & Breytenbach 1985; Curtis & Perrin 1979; Perrin 1980). *Rhabdomys* are diurnal and are active mostly during the morning and evening, avoiding the hottest times of day (Schradin 2006).

Historically the *Rhabdomys* genus of the Muridae rodent family was thought to be monospecific, with only a single species, *Rhabdomys pumilio* (De Graaf 1981). However recent mitrochondrial DNA investigations have revealed the existence of two distinct lineages; *Rhabdomys pumilio* (arid, western part of southern Africa) and *Rhabdomys dilectus* (mesic, eastern part of southern Africa) (Rambau *et al* 2003).

In this thesis, DNA investigations were not carried out on rodents, thus the species of *Rhabdomys* is not specified.

Thesis objectives

- Record and characterise the interaction between *Rhabdomys* and *C.congesta* females (referred to as 'culm-felling' from here on).

- Investigate the effect of culm-felling on myrmecochory (dispersal of seeds by ants) and female *C. congesta* plant canopy structure.

- Perform a detailed physiological study of *C. congesta* sexes looking into nutrient dynamics (in particular, nutrient resorption) and photosynthetic performance in conjunction with different male/female reproductive costs. This included considering the effects of culm-felling by *Rhabdomys* on female physiology by investigating the direct physical effects of culm-felling on female canopies (nutrient loss, micro-climate change).

Thesis Structure

This study includes 3 chapters. The structure and aims of each chapter are summarised below.

- **Chapter 2:** This chapter documented a previously unrecorded interaction between the *Rhabdomys* and female *C. congesta* plants. Using camera trapping technology it was discovered how *Rhabdomys* targeted female plants for their nut-like seeds. The striking side-effects of this interaction on female plant canopies were also quantified. The findings in this chapter can be studied further in the context of dioecism as well as myrmecochory.

- **Chapter 3:** In this chapter nutrient resorption (as a strategy against nutrient limitation) was investigated and compared between *C. congesta* strategies. I investigated the potential for males and females to undertake nutrient resorption and whether resorption was flexible between sexes in response to differing nutrient demands/pressures. Specifically the effect of differing reproductive effort (seeds vs. pollen) between sexes and the loss of pre-senescent plant tissue from female plants during interactions with *Rhabdomys* were investigated. Culm nutrient concentrations were recorded in males and females over time, allowing for the quantification of nutrient resorption. Soil nutrient concentrations were also measured.

- **Chapter 4:** In this chapter I compared the photosynthetic performance of male and female *C. congesta* plants. Isotopes (d13C and d18O) and gas exchange were used to study long-term and immediate photosynthetic performance and moisture dynamics. A number of factors which affect photosynthesis were compared between sexes, including micro-climate (light, soil moisture, temp, humidity, dew point) and phenology. Furthermore I considered the potential effects of the interaction between *C. congesta* females and *Rhabdomys* which could affect micro-climate and nutrient availability through the felling of culms.

CHAPTER 2:

Sex-specific culm-felling in dioecious restios:

The burden of seeds in *Cannomois congesta* females

ABSTRACT

Differing plant canopy structures between the sexes of the dioecious *Cannomois congesta* (Restionaceae) were found to be the result of a destructive seed acquisition strategy used by the four-striped mouse (*Rhabdomys*). Mature, reproductive culms on female plants were felled down (mid way down the culm) in large numbers by *Rhabdomys* in order for it to access the seeds held terminally on culms. In 2014, an estimated 15% of mature, living culms were felled from females during September-November, leading to significant seed losses. Male plants were largely unaffected due to their lack of seeds. Seeds acquired through culm-felling by *Rhabdomys* were all eaten shortly after their discovery, leading to significant seed mortality each year.

Sex-specific culm-felling pressure over a number of years has resulted in female *C. congesta* canopies being dominated by felled, culm-stumps (half culms) and male canopies being dominated by senescent culms. When measured in 2014, females were found to have a mean of 81% of their total culms (ever produced) felled (ratio increases over time), while males had roughly the same proportion of senescent culms. The proportion of living culms was 11% lower in females. Culm diameter was similar between sexes, however females had larger basal diameters on average leading to a higher number of total culms.

The effects of culm-felling on female *C. congesta* canopy structure could have implications for many different interlinking plant processes, leading to differences in overall functionality and fitness between *C. congesta* sexes. The loss of seeds presents a significant challenge for myrmecochory as seeds predated upon by *Rhabdomys* did not make it to the ground, thus excluding ants from the competition for these seeds. This was added to the pressure from *Rhabdomys* for seeds that did manage to fall to the ground. These findings provide the rationale to further investigate the effects of such a sex-specific phenomenon and its incidence in other species.

INTRODUCTION

Within the Restionaceae, a range of seed dispersal mechanisms exist with different groups producing differing diaspore (seed + dispersal structures) types (Linder 1991). This variation in diaspore morphology reflects two different evolutionary strategies for resource use in reproduction and propagation (Caddick & Linder 2002). Large nut-seeded species produce few, large diaspores while capsule and nutlet producing species produce numerous, small diaspores. Seedlings from large diaspores have been shown to grow to a larger size in their first few months of growth, allowing them to better survive their first summer drought. Conversely, seedlings from smaller diaspores are smaller and less likely to survive their first summer drought. These species rely on the sheer number of seedlings produced for sufficient recruitment in the next generation (Caddick & Linder 2002).

In most species, diaspores are not carried away from mother plants by the wind and so plants must rely on other dispersal methods. Myrmecochory is defined as the dispersal of seeds by ants and is a common feature in many ecosystems including Fynbos of South Africa (Lengyel *et al* 2010; Slingsby & Bond 1983; Berg 1975). Myrmecochorous species typically bear large, nut-like seeds which carry a fleshy, lipid body called the 'elaiosome'. Elaiosomes are thought to have evolved to function as a reward for ant dispersers (Lengyel *et al* 2010), who rapidly discover, transport and bury elaiosome-bearing seeds (Bond & Breytenbach 1985). Once buried, ants will eat only the elaiosome and not the thick, smooth-walled seed itself (Bond & Slingsby 1983; Bond & Breytenbach 1985). The removal of the elaiosome by ants does not harm the seed in any way and may even promote increased persistence of seeds in the seed bank due to reduced attraction for pathogens, parasites and vertebrate predators (Bond & Breytenbach 1985; Christian & Stanton 2004). Seed burial is also thought to provide protection against the extreme temperatures experienced during a fire, while promoting their germination (Newton *et al* 2006).

The burial of seeds by ants can provide important protection from rodent seed predation (Bond & Slingsby 1983; Bond & Breytenbach 1985; Christian & Santon 2004). Rodent seed predation is thought to be a strong evolutionary force that has fuelled the evolution to ant-dispersal in many plant species. Rodents can handle and eat seeds much more efficiently than ants, which typically eat the elaiosomes only. They are able to smell the olfactory cues released by elaiosomes and are too attracted to them. Therefore the removal of the elaiosomes by ants may aid in reducing rodent seed predation (Bond & Breytenbach 1985). The level of rodent predation may depend on season and local environment and a variety of other ecological factors.

While seed predation by rodents is commonly addressed in the literature (Heithaus 1981; Bond & Breytenback 1985; Christian & Santon 2004) most studies have focused on the predation of seeds which have been released from plants onto the ground. Few studies have looked into pre-dispersal seed predation and how it can exclude ants from finding seeds and have physical consequences on seed-producing plants (females in the case of dioecious species). Pre-dispersal seed predators can directly reduce plant fitness by increasing seed mortality and damaging reproductive/vegetative structures (Parachnowitsch & Caruso 2008).

Here I investigate an ecological system in mountain fynbos of the Western Cape where a previously unrecorded interaction occurs between the four-striped grass mouse, *Rhabdomys* and the nut-fruited female plants of the dioecious *Cannomois congesta* (Restionaceae). This phenomenon provides us with a unique opportunity to study, *in situ*, the sex-specific effects of pre-dispersal seed predation in restios.

It was recently noticed that the female *C. congesta* individuals were missing many of their mature, seed-baring culms (Figure 1: Left). These culms appeared to have been felled (cut down) off at a fairly consistent height around half way up the culm, leaving many culm-stumps with 45 degree angle points (Figure 2: Left). Culm-fragments (uneaten), seed husks and empty spikelets (which normally enclose the seeds) littered the ground surrounding the female plants (Figure 2: Right). *C. congesta* seed husks were also found in large piles along with rodent dung. From these observations, I hypothesised that the observed patterns are a consequence of rodents seeking the small nut-like seeds borne terminally on female culms. Consistent with this hypothesis, male plants, which lack seeds, did not have their culms felled. Instead, their mature culms senesced, forming a build-up of senescent culm mass amongst the living culms (Figure 1: Right).

Figure 1: Left: Female *C. congesta* with the majority of its culms felled. Right: Male *C. congesta* with the majority of culms senesced.

Figure 2: Left: Recently felled female culm-stumps (still attached to plants) with 45 degree angle points. Right: Fragments of felled female culms surround female plants.

In this chapter I used motion sensing camera-traps and seed-tagging in order to identify *Rhabdomys* as the seed predator affecting *C. congesta* females. I investigated how and when *Rhabdomys* acquired *C. congesta* seeds, and what happened to the seeds once they were acquired. I further explored if there were quantifiable and consistent differences in canopy structure due to culm-felling that might have ecophysiological implications for plant sexes.

Seed predation through culm-felling by *Rhabdomys* is likely to lead to significant reduction in overall ant-dispersal rate for *C. congesta* because seeds taken from plants by *Rhabdomys* before they fall to the ground naturally, excluding ants from interacting with these seeds completely. Furthermore the felling of culms during seed acquisition lead to the striking differences between female and male canopy structures. The loss of culms tissue from females could translate into a number of physiological effects (explored in chapter 3 and 4).

Study site

This study was carried out at Jonaskop where male/female *C. congesta* canopy differences were first noticed (Figure 3).

Figure 3: Map showing the location of Jonaskop (red pin) and surrounding areas Source: Google Earth

Jonaskop is situated in the Riviersonderend mountain catchment at the southern end of the Cape Fold mountain belt. Our study site was located at an altitude of around 900m (-33.941628, 19.524372) on a small plateau on the Eastern side of the service road leading up to the peak. The surrounding vegetation was sparse, low fynbos contrasted by dense stands of mature *Protea repens.* The most recent fires were at least 15 years prior to this investigation and vegetation was mature. The soils at the site were shallow and rocky and are derived from quartzitic sandstone (Agenbag 2006).

METHODS

Recording culm-felling in *C. congesta* females

The initial observation of strikingly different male/female *C. congesta* canopy stuctures at Jonaskop encouraged an investigation into the causes of such patterns. Initial field observations lead us to strongly suspect a rodent seed predator as the cause of this phenomenon (seed husks/rodent dung/bite marks matching rodents). Therefore, starting in August 2014, before any sign of recent culm-felling activity, the terminal inflorescences of selected female *C. congesta* females were baited with a mixture of peanut butter and oats (Figure 4: Left) in order to test the hypothesis that rodents are the cause of the culm-felling phenomenon. Peanut butter and oats have been widely used as bait for attracting rodents. Two inflorescences were baited on each of a number of female *C. congesta* plants, mimicking the reward of an inflorescence holding some very attractive ripe seeds. This was done in order to increase the chances of recording the phenomenon, assuming the rodents would attempt to acquire the bait in the same way they would acquire an inflorescence holding ripe seeds. Baited culms were monitored from late afternoons through to late mornings, using Little Acorn Camera Traps. This experiment was carried out over three day periods each month until December. During these experiments, un-baited female culms were also monitored with camera traps in the hope of catching the phenomenon without the use of bait.

Characterising the culm-felling phenomenon: The fate of seeds

During November 2014, *C. congesta* seeds were collected on site using silk bags. The bags were tied around bunches of mature, female inflorescences on a number of randomly selected female plants. The seeds were collected in the bags as they dropped naturally. Collected seeds were mixed before being used in experiments. Ten seeds were placed out at each of 6 stations beneath restios, 40m apart. These seeds were tagged by gluing (non-toxic) UV threads to the seeds (avoiding the elaiosomes) which allowed seed displacement to be tracked using UV torches after dark. Stations were monitored continuously using Little Acorn Camera Traps (Figure 4: Right). Stations were replenished with tagged seeds after 24hours. This experiment was carried out twice, the 6th-8th November 2014 and 29th-30th December 2014.

Figure 4 Left: Camera trap monitors two culms baited with peanut-butter and oats. Right: Little Acorn Camera Traps monitoring the movement of UV-tagged *C. congesta* seeds

Characterising the culm-felling phenomenon: Plant canopy differences

To quantify the effect of *Rhabdomys* on male/female plant canopies, the proportion of living/senescent/felled culms within the canopy was measured for both sexes. Old generations of culms (felled or senesced) were countable on plants and were thus an indication of the history of culm-felling and senescence in plants. Male and female *C. congesta* plants were measured in 4m * 12m transects. Counting all culms was not possible due to their high numbers and a subjective visual estimate would not have been accurate enough for a detailed comparison of plant morphologies. Thus, a plastic ring with a circumference of 6.5cm was used to sub sample the culms throughout the canopy of each plant (6 sub samples for each plant). In each sub sample, the number of living, felled and senescent culms were carefully counted. The proportion of living/felled/senescent culms was averaged from the 6 sub samples, giving a well integrated proportion of living/felled/senescent culms for each plant. Due to a lack of differentiation between old felling events and recent ones at the time of these measurements, the resultant proportions are integrated values which take into account the felling or lack thereof over a number of years (up until 2014) and are not a measure of yearly felling pressure.

Plant basal area and mean culm diameter were measured using a measuring tape and callipers. For all plants (particularly necessary in males), culms were held together tightly before the circumference at the base of the plant was measured.

Data were analysed using the software package R. Living/felled/senescent culms were a proportion (summed to one), therefore this data could not be analysed using an ANOVA (or non-parametric equivalent) which requires the independence of variables. Instead, data were treated as compositional. The data conversion method followed is described by Van der

Boogaart & Tolosana-Delgado (2006). Following this method, living/felled/senescent proportions were converted into closed compositional data, which were then run through a MANOVA.

To further investigate the sexual differences in individual groups (living, felled or senescent), individual Wilcoxon rank-sum tests were carried out.

In order to confirm that culms were felled and not eaten, the lengths of culm-stumps and felled culm-fragments were added and compared to the lengths of mature standing culms.

Culm number and yearly felling pressure estimates

Total culms/plant were calculated as:

$$\text{Total culms/plant} = 0.83*(R^2/r^2)-1.9 \qquad \textbf{Eqn 1}$$

Where R is plant diameter and r is culm diameter. 0.83 and -1.9 are constants in the equation.

Note: This equation calculates the number of small smaller circles of known dimension (culms) inside a larger circle of known dimension (plant diameter) and was therefore most appropriate for the circular nature of culms and plants. **Eqn 1** was taken from an online forum discussing circle stacking (1). Its results agreed closely to an online circle stacking calculator (2) and was therefore appropriate for our purpose.

(1) (http://math.stackexchange.com/questions/96616/how-many-smaller-circlesradius-is-equal-i -can-fit-within-a-larger-circle.html)
(2) (http://www.engineeringtoolbox.com/smaller-circles-in-larger-circle-d_1849.html)

Using the circle stacking equation, the only variable that is subjective in nature is choosing the space a single culm takes up on the rhizome of the plant. Unfortunately this was not measured so I used double the culm-diameter (measured midway along culm) was used as the space required on the rhizome for each culm because culms were thicker at the rhizome and were not packed together tightly. While total culm number may slightly inaccurate, there was no bias between males and females using this technique and so comparisons remain robust.

In December 2014, at the end of the yearly culm-felling period, freshly trimmed culms were counted around 8 randomly selected females which were visibly affected by the culm-felling phenomenon. It was assumed that the felling activity had come to an end by December as very few mature culms were still attached to plants or were holding seeds by this stage. Freshly felled culms were identifiable due to their fading red/brown colour which contrasted the grey colour of culms trimmed in previous years.

Seed number and losses

The mean number of seeds per culm was calculated by randomly (blind selection) selecting a number of culms with unopened spikelets (which enclose the seeds) from a number of different females throughout the population. Seeds per culm were then counted. This was carried out during seed fall in 2014 (November) in order to make sure seeds had matured and would all be countable. Seed loss due to *Rhabdomys* was estimated using the mean seed number per culm and the mean number of felled culms per female. Both yearly and overall seed losses were calculated in this way.

RESULTS

Culm-felling of female *C. congesta* plants by *Rhabdomys*

Rhabdomys was found to be the rodent species responsible for the culm-felling phenomenon in female *C. congesta* plants. During the evenings and mornings, *Rhabdomys* triggered camera traps. Video footage as well as photographic evidence revealed a fascinating behaviour which allowed *Rhabdomys* to detect seeds/bait at the terminal tips of mature female culms (Figure 5: Top), fell down the culm midway using its teeth (Figure 5: Middle) and reel in the terminal inflorescence containing seeds using its paws, without leaving its perch in the canopy of culms (Figure 5: Bottom). This behaviour explained the pattern of felled culm-fragments sticking out from the side of female plants (Figure 2 Right), as seen in the initial observation period. Inflorescences were sometimes processed underneath restios or were carried away to nearby sheltered sites for processing. Seed processing sites were identified by piles of seed husks, spikelets and rodent dung. Processing of inflorescences involved the extraction and consumption of seeds.

During the period of camera trapping from August to December, the culm-felling phenomenon was only recorded in mid September to November 2014. However all activity during September was associated with the use of peanut butter and oats. Natural, non-baited culm-felling was recorded in October and November. This coincided with the peak seed dropping period of *C. congesta* which appeared to occur over October/November/December (pers. obs.).

Figure 5: *Rhabdomys* acquiring the seeds of a *C. congesta* female. *Rhabdomys* locates an un-baited culm (Top). The culm is felled around the middle using sharp teeth (Middle) and the terminal seeds are pulled in towards the rodent for consumption (Bottom). The terminal inflorescence holding the seeds is circled in red.

Characterising the culm-felling phenomenon: The fate of seeds

Within 24 hours of the start of each UV-tagged seed experiment, a large proportion of tagged seeds had been eaten by *Rhabdomys* (Table 1). Seeds were either eaten at the station or moved (often 2 at a time) to a nearby seed-processing hideout and eaten. This occurred during the mornings and evenings. Tagged seeds moved and eaten by *Rhabdomys* (identified on camera traps) were located at night using UV torches (Figure 6: Top Left). UV powder scattered at stations also helped to track the movement of *Rhabdomys* after it discovered tagged seeds (Figure 6: Top Right). Eaten seeds were found in large processing-hideouts (Figure 6: Bottom Left) and could be identified as eaten through the presence of a seed husk, bitten open with the insides removed (Figure 6: Bottom Right). A small percentage of seeds moved by *Rhabdomys* were abandoned on the open ground, presumably *en route* to a seed processing hideout (Table 1). This could have occurred when tags became snagged during their transport as seen in one video clip captured on camera traps.

Further observations during the day and night showed that ants were highly attracted to the small, white elaiosomes attached to *C. congesta* seeds (Figure 7 white circles) however, specific ant interactions were not investigated.

Figure 6: Fate of *C. congesta* seeds found b*y Rhabdomys pumillio*. Top Left & Top Right: UV tags (found at night) indicate that *Rhabdomys* had moved and eaten seeds underneath nearby bushes (during the eve/morn). Bottom Left: Piles of seed husks visible in protected sites. Bottom Right: Seed husks left over from *Rhabdomys* feeding.

Figure 7: Ants eating elaiosomes during the night.

Table 1: Summary of *C. congesta* seed interactions in November & December 2014. N = 12 stations each holding 10 UV-tagged seeds. (Experiments 1 and 2 combined)

Interaction within 24 hours of placement:	Mean # seeds/station	Std. error	% of total
Eaten on site by *Rhabdomys*	1.0	0.6	10.0
Moved and eaten by *Rhabdomys*	1.7	0.8	16.7
Moved and dropped by *Rhabdomys*	0.3	0.3	3.3
Elaiosomes eaten in night by ants (not buried)	0.8	0.4	7.5
Elaiosomes eaten at day by ants (not buried)	1.1	0.5	10.8
No interaction	5.2	1.1	51.7

Characterising the culm-felling phenomenon: Plant canopy differences

Female and male *C. congesta* plants were differentially affected by culm-felling (Figure 8). In females, the majority of culms had been felled by *Rhabdomys* with very few full culms remaining on the plant long enough to senesce. In males no felling was found. The majority of culms on males had remained attached to plants long enough to senesce and turn completely grey. Thus, sex had a highly significant effect on the overall culm-compositions of males and females Pillai's Trace = 0.83, F (2,53) = 128.6, $p < 0.01$.

The proportions of both felled (W (n1=28, n2=32) = 896, p<0.01 one-sided) and senesced culms (W (n1=28, n2=32) = 0, p<0.01 one-sided) were significantly different and contrasting between sexes.

Interestingly, males had a significantly higher proportion of living culms relative to females W (n1=28, n2=32) =162.5, p<0.01 two sided. The mean relative proportion of healthy culms on males was 11% higher than on females. Living male culms showed much variation on a spectrum from nearly senescent to healthy while most living female culms were green and healthy.

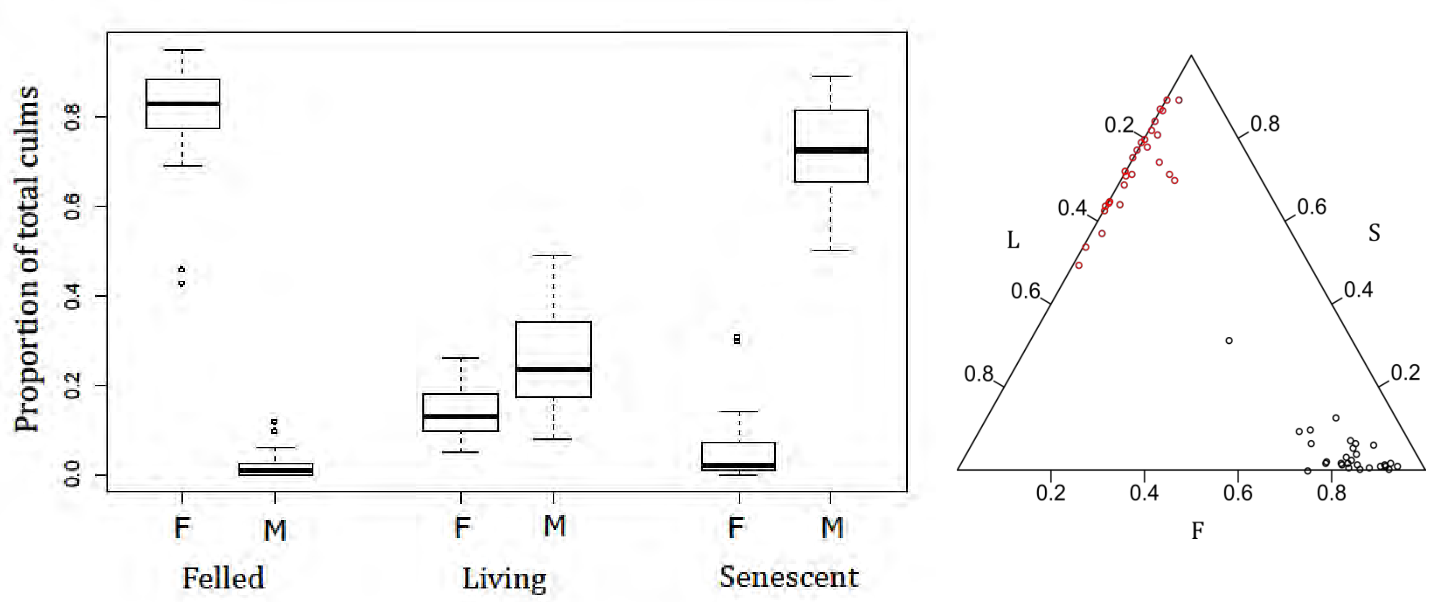

Figure 8: Left: Proportion of living, felled and senescent culms in male and female *C. congesta* plants. Box and whiskers represent the median, inter-quartile range and min/max values. Points represent outliers. Males, N=28, Females, N= 32. Right: Visual representation of living/felled/senescent ratios once converted into compositional data. Red circles = Males, black circles = Females.

After the removal of seeds/inflorescence from culms by *Rhabdomys*, the culm-fragments were left on the ground forming a dense litter around the base of female plants. Remaining culm-stumps appeared to remain photosynthetically active for some time (around a year). The mean lengths of culm fragments surrounding females and remaining culm-stumps were equal and added up to equal the length of un-felled, mature female culms (Figure 9). This indicated that culm tissue was not eaten during the process.

Additionally, comparions between the proportion of felled culms in the centre and on the perimeter of female canopies revealed no significant differences, t(190)=0.38, p = 0.71, meaning that culm-felling occurred homogenously throughout female canopies.

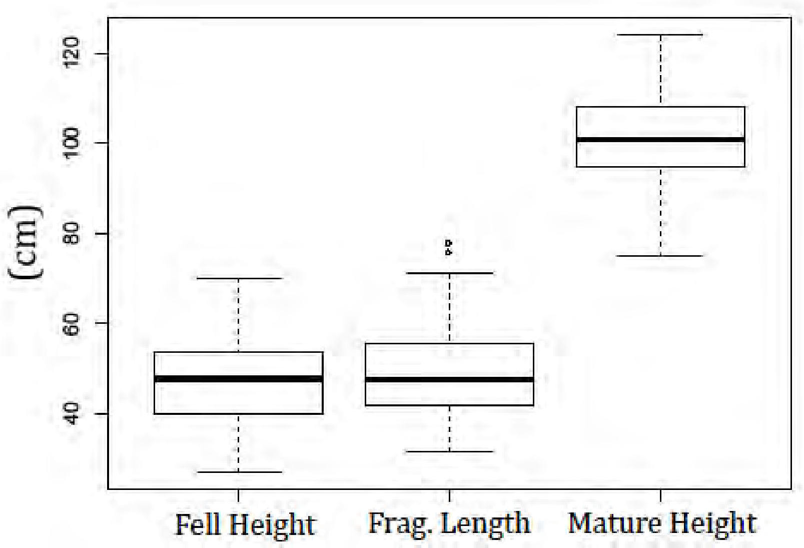

Figure 9: Boxplot representation of the mean height at which culms are felled (FellHeight n=124), the length of felled culm-fragments (Frag. Length n=122) and the mature culm height (MatureHeight n=124) in females. Box and whiskers represent the median, inter-quartile range and min/max values.

Culm number and felling pressure estimates

While culm diameter was not significantly different between sexes, females had significantly wider plant diameters (Table 2). Females therefore had more culms in total (living + felled + senescent) compared to males due to their larger diameter (Table 2).

Table 2: Basic morphological comparisons between *C. congesta* sexes. Mean ± (SE)

	Female	Male	t-test p-value
Basal Plant Diameter (R)	30 (2) cm, n=32	24 (2) cm, n=28	**0.046**
Basal Culm Diameter (r) (mid culm diameter *2 – see methods)	0.41 (0.01) cm, n=8	0.40 (0.01) cm, n=8	0.725
Estimated Total culms (0.83)(R^2/r^2)-1.9	4414 (323)	2900 (268)	n/a

An average of 91 (SE ± 20) culms were felled per female plant in 2014. This seasonal culm-felling pressure was responsible for the loss of an estimated 15% (SE ± 4%) of living culms at the time.

Seed number and losses

All seeds interacted with by *Rhabdomys* were eaten (excluding the few potentially snagged seeds). There was a mean of 4 seeds per culm in females. Therefore, on average, this number of seeds was lost for every culm felled. Using seasonal felling pressure observations (mean of 91 culms felled per female), mean seed loss in 2014 was estimated to be 364 (SE ± 85) seeds per female. Using total estimated culms per female (mean of 4414 culms) and the mean proportion of felled culms in females (81%), the total seed loss since reproductive maturity was estimated to be 13943 (SE ± 1509) seeds per female. These seed losses were equivalent to ~2% and ~ 80% of the total seeds ever produced per female respectively.

Percentage seed loss per yearly reproductive effort could not be calculated because the proportions of reproductive and non(post/pre)-reproductive living culms were not quantified in my data (i.e., culms were counted as "living" up to a year before and after peak seed production, meaning that a functional variety of living culms were present on females at any time). The estimated 15% loss of living culms during 2014 was a measure of culm-loss from a pool of reproductive and non-reproductive living culms. Therefore, because only seed-baring culms were targeted by *Rhabdomys* and because living culms were not all currently producing seeds, it can be assumed that the percentage seed loss per yearly reproductive effort was higher than 15% in 2014.

DISCUSSION

In this chapter, I documented a previously un-recorded phenomenon whereby female *C. congesta* plants, holding nut-like seeds, were heavily targeted by the destructive seed acquisition methods of *Rhabdomys* while male plants were not interacted with. This lead to significant differences in canopy structure between sexes.

Implications of culm-felling for myrmecochory and reproductive output:

All recorded *Rhabdomys* interactions lead to extensive *C. congesta* seed mortality during the study period. Traditionally, it has been shown that rodents (including *Rhabdomys*) and ants compete for seeds on the ground after being released from plants (Bond & Breytenbach 1985; Christian & Stanton 2004). However culm-felling presents a scenario where ants were excluded from such competition because seeds were removed directly from female plants before natural seed-fall. Fewer seeds falling to ground presented fewer opportunities for ant-dispersers to find and bury seeds. This limitation was exacerbated by the continued, rapid predation of seeds by *Rhabdomys* after seed-fall (indicated by UV-tagged seeds placed on the ground), reducing the amount of time remaining seeds spent on the ground for ant-dispersers to find. It has been shown that the ant-dispersal of many Restionaceae species is relatively slow compared to other plant families in Fynbos (Bond & Slingsby 1983), therefore the rapid predation of seeds on the ground by *Rhabdomys* could significantly reduce the number of seeds for ant-dispersers to find. Fewer seeds, spending less time on the ground is a significant limitation for seed dispersal in myrmecochorous plant species. Due to the fact that *Rhabdomys* ate all seeds that they discovered, the likelihood of any rodent-mediated seed dispersal is low regarding this interaction (see Forget 1993; Midgley *et al* 2002 for examples of scatterhoarding). The relative influence of ants versus rodents would be interesting to explore further in the case of *C. congesta* and other nut-fruited restios found to be affected by sex-specific interactions.

Sex-specific effects of culm-felling on plant canopies

In addition to extensive seed mortality, the destructive manner of seed acquisition by *Rhabdomys* was seen to physically alter female *C. congesta* canopies. This lead to sex-specific canopy structures where females had open, felled canopies and males had closed, senescent canopies. The consequential destruction of vegetative plant tissue (ie: where affected vegetative tissue is not targeted directly) has been reported in cases of florivory and pre-fall seed predation (Heithaus 1981; McCall & Irwin 2006). These interactions are usually characterised by vegetative plant tissue being removed as a consequence of seed/fruit/pollen/nectar acquisition by animals. This study is among the first to consider such an interaction in dioecious plants. In this case, the extensive removal of plant tissue only affects large-seeded female plants.

The discovery of a physically destructive method of seed acquisition by rodents provided the rationale to study the physical consequences on the sexes of the dioecious *C. congesta* (See Chapter 3 & 4). Specifically, the removal of culms from female plants and associated canopy differences could be expected to have significant impacts on a number of factors including; 1) nutrient-availability, 2) micro-climate both which influence overall plant physiology.

For example, nutrient resorption has been shown to be important for plant fitness (May & Killingbeck 1992) and has been reported to be high in both restios and grasses with sequential growth (Stock *et al* 1987; Bausenwein *et al* 2001). Thus one might expect a significant loss of important available nutrients from *C. congesta* females due to the large loss in pre-senescent culm biomass (See chapter 3). The removal of culms could also affect micro-climate, changing levels of moisture and light interception. Light and moisture levels are important factors influencing plant physiology by influencing the supply and demand of photosynthesis (Farquhar *et al* 1989) (See chapter 4).

The greater number of culms in female plants could be due to compensatory growth in response the large loss in culms through culm-felling. This has been shown to occur in graminoids in both high and low nutrient environments (Bryant *et al* 1983). Understanding the mechanisms of such growth would require an understanding of the underlying physiological processes. In chapters 3 and 4, I investigate the physiology of *C. congesta* sexes to understand the internal functioning of plants and the effects of both internal and external pressures.

CHAPTER 3

Nutrient resorption and sex-specific nutrient losses in the dioecious *Cannomois congesta*

ABSTRACT

In this chapter I investigated the role of nutrient resorption in Restionaceae within nutrient-poor Fynbos. I measured resorption efficiency (RE) and resorption proficiency (RP) in the dioecious *Cannomois congesta* sexes and investigated whether resorption was flexible in response to the differing nutrient requirements/availability in sexes. Reproductive costs were assumed to be higher in female plants due to the amount of time invested into seed production. Culm-nutrient dynamics in sexes were studied by comparing nutrient concentrations in culms at differing stages of development as well as in soils surrounding plants. Nutrient losses associated with pre-senescent culm loss to *Rhabdomys* (Chapter 2) were estimated using nutrient concentrations and morphological measurements.

Both *C. congesta* males and females were able to undertake high levels of nitrogen and phosphorus resorption, however RE and RP did not differ between sexes. This suggests that nutrient resorption was important in both sexes but was not flexible as a strategy to compensate for the higher reproductive and ecological pressures in *C. congesta* females. Soil nutrients were not different between sexes either, suggesting that females did not compensate through increased soil nutrient uptake. Even without compensatory nutrient resorption or soil uptake in females, there was no evidence of reductions in vegetative growth as expected from increased reproductive requirements and nutrient losses to *Rhabdomys*. This could point to the existence of other compensatory mechanisms (phenology and physiology are investigated in Chapter 4).

Due to the loss of pre-senescent culm tissue from females due to culm-felling by *Rhabdomys*, female plants lost a significant proportion (~23.2% N and ~23.7% P) of potential remaining nutrient resorption from culms after seed release. The effects of this remain speculative, but presumably increase the nutrient stress/vulnerability to nutrient stress in females which already have a higher cost of living.

INTRODUCTION

In Mediterranean-type ecosystems, such as fynbos, soils are generally nutrient poor. Nitrogen (N) and phosphorus (P), the two most critically limiting nutrients for plant growth, occur in concentrations which are amongst the lowest in the world (Kruger *et al* 2012, Stock & Lewis 1986, Witkowski & Mitchell 1987). N and P concentrations in plant biomass are determined by many factors including the balance of their uptake, carbon (C) assimilation, and the losses of C , N and P through turn-over, leaching, exudation, herbivores and parasites (Chapin & Shaver 1989; Aerts & Chapin 2000; Eckstein & Karlsson 2001; Rejmankova 2005).

Among the many strategies employed by plants to deal with low soil-nutrient availability is nutrient resorption preceding senescence. This reallocation/reutilisation of nutrients from aging plant tissue is a common feature of plants (Wright & Westoby 2003) and is an especially important feature for nutrient-conserving plants which occur in nutrient poor soils (Chapin 1980) such as those found within the Fynbos. An experiment by May & Killingbeck (1992) was among the first to test the hypothesis that nutrient resorption contributes significantly to plant fitness. This experiment empirically demonstrated how the defoliation of pre-senescent foliage can lead to long-term, detrimental effects on plant reproduction and growth.

Although many studies have investigated nutrient resorption, a complete understanding of the factors controlling resorption is still lacking (Rejmánková 2005). It has recently been hypothesised that levels of nutrient resorption could reflect the nutritional requirements of plants; with more resorption of nutrients occurring in plants which are more highly nutrient stressed. While studies are few, there is evidence to support this hypothesis showing a significant, negative relationship between levels of nutrient resorption and nutrient availability (Rejmánková 2005; Meyor *et al* 2014; Vourlitis *et al* 2014). This idea could be extended to dioecious species (Pavon & Ramirez 2002), where levels of nutrient resorption could reflect differences in reproductive requirement or nutrient availability between sexes. From this point on, I make the assumption that female reproductive costs are higher than male reproductive costs due to the fact that female culms produced nuts for a full year after male reproductive function was over (see Chapter 4: Figure 9). The assumption of higher reproductive costs in females is well supported (Darwin 1877; Lloyd & Webb 1977; Antos & Allen 1990; Delph 1999; Obeso 2002) and has been made in a number of published studies (eg Harris & Pannell 2010; Barratt & Hough 2012).

The dioecious Restionaceae are responsible for a large proportion of plant diversity in Fynbos and should be more widely investigated in the context of plant strategies against nutrient limitation. While studies are few, there is strong evidence to suggest that nutrient resorption could be an important resource for sequentially growing Restionaceae (new culms growing simultaneously each year) (Stock *et al* 1987; Meney *et al* 1994; Bausenwein *et al* 2001). For example, high levels of nitrogen resorption from senescing culm tissue have been reported in *Thamnochortus punctatus* (*T. punctatus)* (Stock *et al* 1987). Remobilised nitrogen from mature culms of *T. punctatus* played an essential role in alleviating nitrogen limitation for early culm elongation as rhizome activity was low at this time. Similarly, a number of grasses and sedges primarily use remobilised leaf nitrogen for spring growth. New spring leaf growth was found to be largely independent of soil nutrient uptake provided overwintering leaves were present (Bausenwein *et al* 2001). These findings have emphasised the potential importance of nutrient resorption for sequentially growing restioid plants living in nutrient-poor regions.

In this chapter I investigate the nutrient dynamics of *Cannomois congesta* sexes. The higher cost of reproduction as well as the loss of pre-senescent culm-tissue due to culm-felling (see Chapter 2) in females could mean that females are more nutrient-stressed than males and may undertake higher levels of nutrient resorption from available culm tissue as a compensatory mechanism (Obeso 2002). Note: If reproductive costs were in fact equal in males and females (contrary to the assumption made), any physiological differences found between males and females could not be related to different overall reproductive costs. Rather, sex-specific, external influences and differences in the timing of reproductive effort would provide a better explanation for any physiological differences found.

This chapter addresses the following questions:

1) To what degree does nutrient resorption occur in *C. congesta* sexes?
2) How much nutrients are lost from female *C. congesta* plants through culm-felling by *Rhabdomys* (Chapter 2)?
3) Are levels of nutrient resorption flexible between *C. congesta* sexes in response to differences in nutrient availability/losses and reproductive allocation?
4) Do female *C. congesta* compensate for higher reproductive allocation and nutrient losses to *Rhabdomys* through increased soil nutrient uptake? Increased soil nutrient uptake has been shown to be stimulated by nutrient deficits. (Marx *et al* 1977, Chapin & Slack 1979, Chapin 1980, Waring & Schlesinger 1985).

To investigate these questions, culm nutrient concentrations in male and female *C.congesta* plants were compared over a range of developmental stages in the culm life-cycle. This allowed for culm nutrient concentrations as well as the timing and degree of nutrient resorption to be assessed and compared between the sexes. Soil nutrient concentrations were also recorded in both sexes.

METHODS

Culm samples for nutrient analysis

A number of mature/healthy culms were excised from each of 6 males and 6 females which were spaced apart widely and had little surrounding vegetation. This sampling was carried out twice (April 2014 and November 2014). A number of fully senescent culms were also excised from each of these plants. Only culms which had turned completely grey in colour, were undamaged and still attached to plants were classified as senescent. It was not possible to identify the exact stage of senescence in culms and so three samples per plant were carefully selected and used in order to get the best possible values. Culms were cut off at the base as close to the rhizome as possible. Both the living and senesced culms (n=6) collected in November 2014 were separated into upper and lower culm-region by cutting culms in half. This was done to better understand the distribution of nutrients within culms. Culm samples were finely ground using a mill. Culm nitrogen concentrations were determined using a Nitrogen Analyser (Leco), total combustion method. Phosphorus concentrations were determined using Nitric/ Hydrochloric total Acid digestion, ICP – OES (Inductively coupled plasma optical emission spectroscopy) elemental analysis. All analyses were carried out at BEMLAB.

Soil Samples for nutrient analysis

Three auger loads of soil were collected in a triangular pattern around each of 9 males and 9 females, taking care to sample close to the rhizome. Soil samples were mixed well and sieved using a 2mm mesh diameter. Samples were then air dried for 2 weeks prior to nutrient analysis. Soil nitrogen concentrations were determined using the Leco total combustion method. Phosphorus concentrations were determined using the Bray II extract test using ICP – MS (Inductively coupled plasma mass spectrometry).

Calculating nutrient resorption

To quantify levels of nutrient resorption, resorption efficiency (RE) and resorption proficiency (RP) were investigated. RE was calculated using the equation proposed by Killingbeck & Costigan (1988).

$$\mathrm{RE}\ (\%) = [(\mathrm{N}_{\text{mature leaves}} - \mathrm{N}_{\text{senescent leaves}})/(\mathrm{N}_{\text{mature leaves}})] \times 100$$

RP is simply the nutrient concentration left over after complete senescence. This is a measure of the success of resorption as a nutrient conservation strategy in plants (Killingbeck 1996). The mean dry mass of culms did not change between different sampling times (April, November &

senescence) which means nutrient resorption values (RE and RP) were not affected by changes in culm dry mass.

Calculating nutrient losses

To quantify nutrient losses in female *C. congesta* plants due to culm-felling by *Rhabdomys* (see chapter 2), Eqn1 and Eqn2 were used. To obtain the total percentage nutrients lost, the result of Eqn2 was divided by that of Eqn1 and multiplied by one hundred.

Total potential nutrient resorption (without culm-felling) **Eqn1**
= % nutrient (April) * total culm weight * % resorbed (until senescence)
* mean # culms/plant

Total potential nutrient resorption losses (due to felling) **Eqn2**
= % nutrient (November) * fallen culm weight * % resorbed (until senescence)
* mean proportion felled * #culms/plant

Total culm weight was obtained by weighing full culms excised from female plants. The lengths of excised culms were also measured to get mean mass/cm in healthy culms. This was done for the top and bottom sections separately of culms because culms may be thicker at the bottom. Fallen culm weight (culms felled down by *Rhabdomys)* was calculated by measuring the length of felled culm fragments and multiplying by mass/cm of upper culms (see Table 1 for values).

Table 1: Culm weight and culm number means used in estimations of female nutrient losses

	Mean total culm weight (g)			Mean fallen culm weight (g)	Mean # culms / plant (Chapter 2)
	Full	Bottom	Top		
Mean	2.88	1.7	1.18	0.98	4414
SE	0.09	0.08	0.04	0.05	323

The mean percentage of female culms felled is 81 % (see chapter 2). Percent resorption is taken from data displayed in Table 2.

Statistical considerations

The software package R (R Development Core Team 2008) was used to analyse data. Nutrient concentrations were compared between sexes and over time using general linear models (GLMs). Upper/lower culm nutrient comparisons as well as soil nutrient concentrations between the sexes of *C. congesta* were analysed using multiple Wilcoxon rank-sum (Mann-Whitney-Wilcoxon) tests. Non-parametric tests were used because the data violated the assumption of normality in a number of cases when sample size was small. The results of non-parametric tests were compared against the results of parametric tests and in no cases were there any marked differences.

RESULTS

Culm nutrient concentrations and resorption

Culm development stages included the first measurements made in April 2014 (early seed production), November 2014 (Seed release) and fully senesced (completely grey and brittle). Both nitrogen and phosphorus concentrations (Table 2) were not significantly affected by sex, while culm development stage had a highly significant effect (Table 3 & 4).

Table 2: Culm nutrient (mean upper + lower) (N & P) concentrations in *C. congesta* males and females at different culm developmental stages (April, November, fully senesced). Resorption efficiency (RE) relative to the earliest measurements in April is displayed. Mean ± (SE). (*) Note: culm nutrient concentrations before April were not measured and therefore we do not know if any resorption had taken place earlier.

Culm development stage		Mean culm-tissue N (%)	% RE (N) (relative to April)	Mean culm-tissue P (%)	% RE (P) (relative to April)
Males					
April 2014		0.55 (0.03)	*	0.02 (0.003)	*
November 2014		0.43 (0.03)	22.7 (2.1)	0.01 (0.001)	42.3 (7.9)
Fully senesced		0.30 (0.013)	46.6 (3.5)	0.002 (0.001)	92.3 (66.6)
Females					
April 2014		0.60 (0.03)	*	0.025 (0.002)	*
November 2014		0.43 (0.02)	28.5 (1.7)	0.014 (0.001)	43.3 (6.1)
Fully senesced		0.35 (0.03)	41.7 (4)	0.009 (0.003)	64.4 (22.4)

Table 3: Results of a general linear model (GLM) testing nitrogen (N) concentrations against explanatory variables, ‘sex’ (male/female) and ‘culm development stage’ (April, November, fully senescent).

Residuals:	Min	1Q	Median	3Q	Max
	-0.123333	-0.034583	-0.007083	0.036667	0.178333
Coefficients:		Estimate	Std. Error	T value	Pr(>|t|)
	Intercept – Female April	0.603333	0.027765	21.730	< 2e-16 ***
	November	-0.171667	0.039265	-4.372	0.0001 ***
	Fully senescent	-0.251667	0.035844	-7.021	3.08e-08 ***
	Male	-0.050000	0.039265	-1.273	0.2110
	November: Male	0.045833	0.055529	0.825	0.4146
	Fully senescent: Male	-0.006111	0.050691	-0.121	0.9047

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 0.06801 on 36 degrees of freedom
Multiple R-squared: 0.746, Adjusted R-squared: 0.7107
F-statistic: 21.15 on 5 and 36 DF, p-value: 8.144e-10

Table 4: Results of a general linear model (GLM) testing phosphorus (P) concentrations against explanatory variables, ‘sex’ (Male/Female) and ‘culm development stage’ (April, November, fully senescent).

Residuals:	Min	1Q	Median	3Q	Max
	-0.011667	-0.002500	-0.001667	0.002500	0.021111
Coefficients:		Estimate	Std. Error	t value	Pr(>|t|)
	Intercept Female – April	0.025000	0.002435	10.268	3.05e-12 ***
	November	-0.010833	0.003443	-3.146	0.00331 **
	Fully senescent	-0.016111	0.003143	-5.125	1.02e-05 ***
	Male	-0.003333	0.003443	-0.968	0.33949
	November: Male	0.001667	0.004870	0.342	0.73415
	Fully senescent: Male	-0.003889	0.004445	-0.875	0.38748

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 0.005964 on 36 degrees of freedom
Multiple R-squared: 0.6725, Adjusted R-squared: 0.627
F-statistic: 14.78 on 5 and 36 DF, p-value: 6.836e-08

Because culm nutrient concentrations were not significantly different between sexes at any stage, estimates based directly on these concentrations (i.e resorption efficiency) did not have any significant difference either. This was confirmed using two-sample t-tests which found no significant differences in resorption efficiency across sexes.

Heterogeneity of nutrient concentrations and resorption along the length of culms

Nitrogen was significantly more highly concentrated in the upper culm-region of both sexes while phosphorus showed this pattern only in females (Table 5).

Table 5: Comparison of nitrogen and phosphorus concentrations in the upper and lower regions of healthy male and female *C. congesta* culms sampled in November 2014. Values represent the median (n=6) nutrient concentrations in culms. Inter-quartile range (IQR) included in brackets for each nutrient concentration. The Wilcoxon W-stat and associated p-value is displayed for each male vs. female nutrient comparison.

November 2014 Samples		UPPER	LOWER	UPPER vs. LOWER	
		Median (IQR) % in culm tissue	*Median (IQR)* % in culm tissue	Wilcoxon p-value (two-tailed)	W-stat
Male	Nitrogen	0.54 (0.04)	0.32 (0.06)	**0.008**	1
	Phosphorus	0.015 (0.01)	0.01 (0)	0.071	9
Female	Nitrogen	0.53 (0.06)	0.31 (0.02)	**0.005**	0
	Phosphorus	0.02 (0.01)	0.01 (0)	**0.027**	6

% resorption of N and P was generally higher in the upper-culm region of sexes however this was only significant for N in males (Table 6).

Table 6: Comparison of % resorption efficiency (RE) of nitrogen & phosphorus between upper and lower culm-regions in *C. congesta* sexes (n=6 for each group). These values represent RE between November and full senescence.

		UPPER	LOWER	UPPER vs. LOWER	
		Median (IQR) *% RE*	*Median (IQR)* *% RE*	Wilcoxon p-value (two-tailed)	W-stat
Male	Nitrogen	36.2 (13.9)	12.5 (24.4)	**0.0411**	5
	Phosphorus	100 (0)	100 (0)	0.405	21
Female	Nitrogen	40 (10.5)	15 (21.8)	0.065	6
	Phosphorus	100 (37.5)	0 (75)	0.181	10

N & P losses in female C.congesta due to culm-felling by Rhabdomys

Using nutrient concentrations and morphological measurements, the total (since reproductive maturity) estimated nutrient resorption of a theoretical female plant with no felling was calculated (Table 7). These estimates represent the potential resorption since reproductive maturity that females could have without pre-senescent culm loss to *Rhabdomys*. To compare to this, the estimated overall nutrient losses since reproductive maturity due to culm-felling were calculated. These losses due to *Rhabdomys* are displayed in Table 7 and account for an overall loss of 23.2% and 23.7% of the total potentially resorbed N and P, respectively.

Table 7: Mean nitrogen and phosphorus losses from female *C. congesta* females due to culm-felling by *Rhabdomys*. Nutrient concentrations in the female upper culm-region were used in the nutrient loss estimates because culm-felling results in the loss of upper culms.

Nutrient (g)	Total potentially resorbed nutrients/plant (without culm-felling)	SE	Total losses/plant	SE
Nitrogen	31.74	4.27	7.36	1.18
Phosphorus	2.03	0.74	0.48	0.14

Soil nutrients

Nitrogen and phosphorus concentrations in soils were not significantly different between sexes (Table 8).

Table 8: Soil nutrient concentrations surrounding male and female *C. congesta* plants. Medians displayed with inter quartile range in brackets. Wilcoxon W-stat and associated p-value is displayed for each male vs. female comparison.

	MALE	FEMALE	M vs. F	
Soil Nutrient	*Median (IQR)*	*Median (IQR)*	Wilcoxon p-value (two-tailed)	W-stat
% Nitrogen	0.06 (0.01)	0.06 (0.01)	1	18
Phosphorus (Bray II)	1 (0.75)	2 (0.75)	0.311	24

DISCUSSION

To what degree does nutrient resorption occur in C. congesta?

Reallocation of nutrients from mature culm-tissue did occur in both male and female *C. congesta* plants, as shown by the highly significant decrease in culm nutrient concentrations between April, November and full senescence. This suggests that nutrient resorption continued in culms until full senescence, occurring during and after sexual function in both male and female culms.

Resorption efficiency (RE) was within the expected levels for nutrient conserving restios supporting the findings of Stock *et al* (1987), Meney *et al* (1988) and Bausenwein *et al* (2001) which showed the prevalence and potential importance of nutrient resorption in rush–like plants in nutrient limited regions. Furthermore, resorption proficiency (RP) values of around 0.3% N and less than 0.01% P (see fully senescent culm nutrient concentrations in Table 2) revealed that resorption was occurring close to the maximum possible level in both sexes. RP is defined by Killingbeck (1996) as the lowest possible nutrient concentrations in the senescent foliage of woody perennials (Killingbeck 1996). Values of below 0.7% N and 0.05% P indicate highly proficient resorption while values around 0.3% N and 0.01% P are thought to represent senescence which is biochemically complete (Killingbeck 1996).

The earliest nutrient concentration measurements were only taken after flowering, during the start of early seed production in females (April 2014). Therefore we cannot determine when the resorption of nutrients initially commenced, only that it did occur between the time of early seed production, seed fall and full senescence (between post-flowering and full senescence in males).

How much nutrients are lost from female C. congesta plants through culm felling by Rhabdomys (Chapter 2)

Pre-senescent culm removal (culm-felling) by *Rhabdomys* (Chapter 2) presented a situation where both seeds and culm tissue were removed from female plants at the time when seeds had fully matured (very beginning of seed fall coincided with culm-felling). Seeds lost to *Rhabdomys* represented significant resource losses from females. However nutrients allocated to seed were lost from female plants whether seeds were eaten or not. Due to the loss of culm tissue before complete senescence, nutrients remaining in the top half of female culms after seed maturation (which would otherwise be reallocated to new culm tissue) were lost. Whether this presented an immediate challenge for female plants is unclear. Remaining culm-stumps left over from felling events could provide significant nutrient inputs for females after a felling event. This could be sufficient to prevent any major reductions in plant fitness. However it is possible that small reductions each year will eventually have marked effects on overall plant fitness such that growth and reproduction are reduced significantly by nutrient limitation. To quantify the effect of culm-felling by *Rhabdomys*, experimentation would have to be carried out on felled vs. non-felled females using exclusion experiments. Other experiments could also involve manipulating reproductive investment in order to study the effects on growth (as shown in Alvarez-Cansino 2010).

Are levels of nutrient resorption flexible between C.congesta sexes in response to differences in nutrient availability and reproductive allocation?

Female *C. congesta* investment of nutrients into seeds began shortly after flowering (detectable by the rapid swelling of female inflorescences) and continued until seed release a full year later. In contrast, male reproductive function ended after pollen production during flowering. While the nutrient concentrations/dry mass of reproductive parts were not recorded in either sex, we assume that female reproductive cost was higher overall due to the long duration of seed development compared to pollen production (between 6 - 12 times longer) (Chapter 4: Figure 9). This pattern is generally well supported in the literature (Darwin 1877; Lloyd & Webb 1977; Antos & Allen 1990; Delph 1999; Obeso 2002). It is possible that overall reproductive effort was similar in males and females, although during the time of culm sampling, it is very likely that seed-producing female culms were investing more into reproduction than the post-reproductive male culms.

Between culm maturity (~ April) and seed release (November) in females, culm-stored nutrient concentrations as well as levels of resorption were no different to males. This suggests that at the culm-level, resorption was not flexible between the *C. congesta* sexes in response to differing reproductive requirements during this time (Rejmánková 2005; Pavon & Ramirez 2008; Meyor *et al* 2014; Vourlitis *et al* 2014). RP values suggested that resorption was already occurring at (or at least close to) the physiological maximum in both sexes (Killingbeck 1996) and therefore compensation for potentially higher reproductive costs/nutrient losses in females presumably could not be achieved through further increases in resorption. Resorption appeared to be constant across the sexes regardless of the differences in the timing of reproductive effort. It is more likely that allocation of resorbed nutrients was flexible between sexes. Resorbed nutrients from male culms could have been used to initiate the earlier elongation of the next generation of culms while females focused on seed production (see Chapter 4, Figure 7).

Because increased resorption was not evident as strategy in females to compensate for potentially higher reproductive costs or nutrient losses, females could experience costs in fitness such as reductions in vegetative growth or future reproductive output (Alvarez-Cansino

2010; May & Killingbeck 1992), unless other compensatory mechanisms/adaptations were at present (Pavon & Ramirez 2008).

Evidence for a reduction in vegetative growth in females is limited. Culm dimensions were no different between sexes and females were actually larger in circumference (Chapter 2). Unfortunately the density of new culm growth each year was not recorded in either sex, making it difficult to compare growth between them. If no differences were detected, this could indicate compensatory mechanisms/adaptations which allow females to reduce/mask the impact of higher reproductive costs. This situation is common in plants (Dawson & Bliss 1989; Dawson & Ehleringer 1993; Obeso 2002; Pavon & Ramirez 2008) because males and females must coexist in order to survive. These compensatory methods could include changes in habitat use (sex-partitioning), phenology and physiology. The *C. congesta* population at Jonaskop occur on a homogenous plateau and so we do not expect to see partitioning between sexes in the habitat, however differences in phenology and physiology are recorded and discussed in chapter 4.

Unfortunately without a previous record of seed production, effects of reduced nutrient availability on future female reproductive output remains speculative. Rhizome size was also not investigated due to the destructive nature of rhizome sampling.

Do female C. congesta compensate for higher reproductive cost/nutrient-losses to Rhabdomys through increased soil nutrient uptake?

Uptake from soils around *C. congesta* sexes was not different as soil nutrient concentrations were similar around male and female plants. Therefore increased soil nutrient uptake was not a strategy in females in order to compensate for increased reproductive costs/nutrient losses (Marx *et al* 1977; Chapin & Slack 1979; Chapin 1980; Waring & Schlesinger 1985). Soil nutrient uptake was probably limited to certain periods where both sexes took advantage equally. This further suggests that male and female plants were efficient enough at conserving nutrients through remobilisation that soil nutrient additions over time were sufficient to build up a positive balance of nutrients, even with additional reproductive costs & losses of culm tissue in females.

In summary, reabsorbed nutrients presumably made up a significant component of available nutrients for male and female *C. congesta* plants due to the fact that nutrients were resorbed highly efficiently/proficiently in both sexes. However females did not appear to be able to compensate for higher reproductive costs/nutrient losses through increased resorption, probably due to resorption already occurring at its physiological maximum. Fitness costs in females were not detected, potentially due to other compensatory mechanisms.

CHAPTER 4

Photosynthesis, micro-climate, phenology and the effects of sex-specific culm-felling in the dioecious *Cannomois congesta* (Restionaceae)

ABSTRACT

Using stable isotopes ($\delta^{13}C$ & $\delta^{18}O$) and gas exchange, I compared the photosynthetic performance of male and female *C. congesta* plants in order to understand the effects of greater reproductive costs in female plants. I further investigated the potential influence of 1) sex-specific micro-climate (due to culm-felling by *Rhabdomys*) and 2) phenology on plant photosynthesis.

Mature culm $\delta^{13}C$ signals were found to be significantly lower in females (-27.86 ± SE 0.13 ‰) compared to males (-27.28 ± SE 0.13 ‰). This small difference was seemingly related to phenological differences in the timing of new culm growth and was not likely to translate into fitness consequences. Gas exchange revealed that the A/g ratio (intrinsic WUE) in mature culms was flexible over time but not different between sexes. Modelled photosynthetic parameters in mature culms also revealed no significant differences between sexes suggesting that photosynthetic performance/functionality was similar at the biochemical level.

Plant canopy micro-climate differences did not clearly reflect the expected effects of the sex-specific canopy structures caused by *Rhabdomys*. Open, felled female canopies did not intercept significantly more light as hypothesised. Moisture differences were found with female soils being more saturated in dry periods however other factors could have influenced these values. Mature culm $\delta^{18}O$ signals suggested, with more accuracy, that plant water status throughout culm growth in males and females were equivalent.

Overall, the general lack of evidence suggesting physiological compensation for higher reproductive costs in female *C. congesta*, as well as a lack of visible fitness costs suggests that females were highly efficient at surviving in their habitat. Phenological plasticity between sexes could contribute significantly to the ability of each sex to use their available resources efficiently, by avoiding conflicts between supply and demand over time. Additionally, the use of rhizome-stored nutrients (not tested) could have acted as a short-term solution to compensate for lost culm-tissue in females (Bryant *et al* 1983; Hopkinson & Schubauer 1984), however this would presumably lead to increased vulnerability to environmental pressures in the long-term.

INTRODUCTION

In dioecious plants, physiological performance can vary in response to differing costs of reproduction between sexes. These responses can be very specific to the particular needs of males and females within a population, depending on their requirements and resource availability (Retuerto *et al* 2000). Such physiological changes are often coupled by habitat partitioning between sexes because physiological specialisation to differing habitats is thought to act as a mechanism whereby males and females can best meet their differing reproductive requirements (Dawson & Bliss 1989; Dawson & Ehleringer 1993). For example, sex-specific differences in stomatal conductance to water vapour (g), Nett assimilation of CO_2 (A) and instantaneous/long term water use efficiency, were able to explain female-biased sex ratios in streamside habitats and male-biased sex ratios in non-streamside habitats in terms of gender specialisation (Dawson & Ehleringer 1993). Other factors such as morphological dimorphism (Harris & Pannel 2010), or phenological plasticity (Delph 1990; Milla *et al* 2006) also compensate for differences in reproductive costs by allowing plants to best make use of the resources around them, even when habitat partitioning is not present. If no physiological changes or specialisations occur which compensate for differing reproductive requirements, certain tradeoffs can occur leading to reductions in photosynthetic capacity (Wheelwright & Logan 2004), growth, reproduction and survivability (Obeso 2002).

The use of gas exchange in combination with stable isotopes has revolutionised the study of dioecism in plants because these techniques provide instantaneous, long-term and integrated measures of photosynthetic performance. These performance differences are important to consider in conjunction with resource allocation because they determine the performance of plants in their habitat, and can translate to differences in whole-plant fitness (Retuerto *et al* 2000). Gas exchange techniques allow for instantaneous measures of the fundamental processes of plant photosynthesis, namely the rate of CO_2 assimilation (A) and stomatal conductance to water vapour (g). The ratio between A/g is also a useful measure because it describes the intrinsic water use efficiency of plants (carbon gained to water lost). A/g ratios also have a known effect the internal concentration of CO_2 inside the stomatal air spaces (c_i) which in turn is translated into predictable differences in the $\delta^{13}C$ signals stored in plant tissue over time through the process of fractionation. Therefore $\delta^{13}C$ signals reflect integrated foliage WUE patterns because they are a measure of the combined average c_i values throughout foliage growth. Furthermore, using a combination of $\delta^{13}C$ and $\delta^{18}O$ can reveal if $\delta^{13}C$ differences are more likely to be related to changes in A or g (Scheidegger *et al* 2000).

Plant A and g can be affected directly by a number of factors. The effects of foliage nutrient concentrations, moisture and light availability on photosynthesis are most commonly studied

(Dawson & Bliss 1989; Dawson & Ehleringer 1993; Sparks & Ehleringer 1997; Retuerto *et al* 2000). Nitrogen concentrations are strongly correlated with photosynthetic rate and capacity because the photosynthetic machinery of plants is largely composed of nitrogenous compounds (Sage & Pearcy 1987; Evans 1989; Sparks & Ehleringer 1997). Moisture and light can both affect photosynthetic water use efficiency by manipulating the rates of A and g (Farquhar *et al* 1989; Retuerto *et al* 2000). Genetic factors can also play role in determining the flexibility of A and g, perhaps because of differences in the biochemistry of photosynthetic machinery (Dawson & Bliss 1989).

In this chapter, I investigated the photosynthetic performance of *C. congesta* sexes using $\delta^{13}C$ and $\delta^{18}O$ signals in combination with gas exchange. The influence phenology, patterns of reproductive effort, culm nutrients (Chapter 3) and sex-specific canopy structure (caused by *Rhabdomys* – Chapter 2), were included in the interpretation of isotope and gas exchange results. The sex-specific canopy structures caused by *Rhabdomys* could lead to microclimatic differences within in female and male *C. congesta* canopies due to possible differences in self-shading (Caldwell *et al* 1983; Setter *et al* 1997) and moisture interception (Marloth 1903; Mol & Romoff 1983), potentially affecting rates of A and g. Increased light penetration could be expected in felled females compared to the un-felled males due to relatively open canopies causing a possible reduction in self-shading. Moisture interception is harder to predict because while females have larger diameters than males (Chapter 2), it is not known if felled culm stumps intercept moisture as well as un-felled, senesced culms.

Unlike similar studies, the *C. congesta* population at Jonaskop occurred in a homogenous, flat landscape with no obvious features that could promote habitat partitioning between sexes (such moisture/soil nutrient gradients etc). Males and females also had similar nutrient concentrations (Chapter 3: Table 2) which leads to the expectation of similar photosynthetic capacity (Sage & Pearcy 1987; Evans 1989; Sparks & Ehleringer 1997). The lack of evidence for physiological changes or specialisations as well as the lack of evidence for reductions in photosynthetic capacity, growth and reproduction could point to other compensatory mechanisms which allow females to survive equally well in the environment compared to males.

METHODS

C. congesta culm sampling and cellulose extraction for carbon and oxygen isotope analysis

Twelve females and twelve males were selected based on the degree of isolation they experienced from one-another and other and competing plants. Individuals with relatively little surrounding vegetation were selected in order to minimize plant-plant interference. At each plant, 3 healthy (dark green and fleshy) culms were excised at the base (as low down as possible). The culms were separated into upper and lower culm-regions and stored in brown paper bags. Standing, grey, senesced culms were also excised from each plant.

Culms were dried out over 48 hours at low heat (<60degrees Celsius) and ground to a fine powder using a ball mill. Care was taken to clean milling equipment between samples to avoid contamination.

Powder from the lower and upper living culms of males and females was used in a Carbon isotope analysis. Samples were weighed into tin cups to an accuracy of one microgram on a Sartorius micro balance. The cups were then squashed to enclose the sample. The samples were combusted in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit. All three items are made by Thermo Scientific, Bremen, Germany. The in-house standards used were: A proteinaceous gel (Merck) and dried and thoroughly homogenised lentils.

All the in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Carbon is expressed in terms of its value relative to Pee-Dee Belemnite.

In three males and three females, culm samples were separated into 3 sections; bottom, middle and top. Holo-cellulose was extracted from each section following the methods in Brendel *et al* (2007), Leavitt & Danzer (1993), and Gaudinski *et al* (2005).

Cellulose oxygen isotope analysis was conducted at ISO Analytical. Oxygen isotope analysis was conducted by EA-IRMS. Total conversion of sample material was performed at 1080 °C in a quartz reactor tube lined with a glassy carbon film, filled to a height of 170 mm with glassy carbon chips and topped with a layer of 50% nickelised carbon (10 mm deep). Carbon monoxide and nitrogen produced were separated on a GC column packed with molecular sieve 5A at a temperature of 65 °C. The IRMS used was a Europa Scientific 20-20 with triple Faraday cup collector array to monitor the masses 28, 29 and 30.

In preparation for oxygen isotope analysis, samples were weighed (1.0 ± 0.1 mg) into silver capsules (8 x 5 mm). The unsealed capsules containing cellulose were placed into the wells of a microtiter plate. The microtiter plates containing batches of 20 samples were placed in a laboratory oven at 60 °C to dry for 10 days. The standards and check samples allocated to each sample batch were weighed and dried together with the cellulose samples for that batch. Once drying was complete sample batches were only removed from the oven and the capsules sealed immediately prior to analysis to minimise the resorption of moisture by the samples.

The reference material used for $\delta^{18}O$ analysis of cellulose samples was IA-R006 (cane sugar, $\delta^{18}O_{\text{V-SMOW}}$ = 35.23 ‰). For quality control purposes test samples of IA-R006, IA-R005 (beet sugar, $\delta^{18}O_{\text{V-SMOW}}$ = 30.84 ‰), IAEA-CH-6 (sucrose, $\delta^{18}O_{\text{V-SMOW}}$ = 36.40 ‰), IAEA-C-3 (cellulose, $\delta^{18}O_{\text{V-SMOW}}$ = 32.20 ‰) and IAEA-601 (benzoic acid, $\delta^{18}O_{\text{V-SMOW}}$ = 23.30 ‰) were measured as quality control checks during analysis of the samples. Silver capsules containing reference and check samples were left open and, with the exception of IAEA-601 (benzoic acid), oven dried along with samples at 60 °C for 10 days in a laboratory oven directly prior to analysis, to remove moisture. IAEA-601 was excluded from drying, as it is susceptible to decomposition upon heating.

IA-R005 and IA-R006 are in-house standards calibrated and traceable to IAEA-CH-6. IAEA-CH-6 and IAEA-C-3 are inter-laboratory comparison standards distributed by the International Atomic Energy Agency for which there are generally agreed $\delta^{18}O$ values. IAEA-601 is an inter-laboratory comparison standard distributed by the International Atomic Energy Agency with an internationally accepted $\delta^{18}O$ value.

Reporting isotope results (notation)

Leaf carbon and oxygen isotope ratio are expressed in delta notation as:

$$\delta^{13}C_{\text{sample}}\ (= \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) * 1000\ ,$$

Or

$$\delta^{18}O_{\text{sample}} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) * 1000\ ,$$

where $\delta^{13}C_{\text{sample}}$ and $\delta^{18}O_{\text{sample}}$ are the isotope ratio in parts per mil (‰), R_{sample} is the 13C/12C or 18O/16O molar abundance ratios of the leaf material and R_{standard} is the accepted standard; PDB (for carbon) and VSMOW (for oxygen) . GLMs were used to study the effects of “sex” and “culm-region” on carbon and oxygen isotope values.

Gas exchange measurements

Net carbon assimilation (A) and stomatal conductance (g) and the internal concentration of CO2 in stomata (c_i) were measured at differing light intensities in male and female plants in 2015 using the Li-Cor 6400 with a flourometer head and CO2 mixer attached. Light was measured as the Photosynthetic Photon Flux Density (PPFD) ($\mu mol/m^2/s$). During measurements, CO2 concentration was fixed at 400ppm, block temperature at 25degrees Celsius and relative humidity between 60 and 70 %. Early morning measurements were made at the felling-line, midway along the culm.

Additional photosynthetic parameters were modelled from data following the methods outlined in Lobo *et al* (2013). This method uses equation (1) (Lambers *et al* 2008), allowing for fundamental photosynthetic parameters to be extracted from the model. The model works using the solver function in Microsoft excel and altering parameters in order to minimize the sum of square differences between measured assimilation and modelled assimilation. These parameters are: Rd (dark respiration), φ (Quantum Yield), Amax (Maximum assimilation rate), θ (Curvature Factor) (See Lobo *et al* 2013). Models were based on the measurements made on 2 females and 2 males.

$$(1) \qquad A_{\mathrm{n}} = \frac{\phi I + A_{\max} - \sqrt{\{(\phi I + A_{\max})^2 - 4\ \Theta\ \phi_{\max}\}}}{2\Theta} - R_{\mathrm{d}}$$

A and g gas exchange values from mature culms in 2012-2014 (Skelton 2014) were used as supplementary material to gain a long-term perspective of physiological variation in A and g in response to changing seasons. The ratio of A/g represents the intrinsic WUE in terms of the rate carbon gained to stomatal conductance (Seibt *et al* 2008). Because the ratio of A to g determines c_i, $\delta^{13}C$ and A/g values should be positively correlated (Farquhar *et al* 1989).

Multiple t-tests were used to compare male and female values for both instantaneous and modelled photosynthetic parameters. Multiple t-tests were also used to compare monthly A/g ratios between sexes.

Microclimate

To measure light interception, Li-Cor quantum PAR (Photosynthetically active radiation) sensors were placed at the felling-line within a female plant canopy and another in the equivalent position within a male canopy. For soil moisture measurements, Campbell Scientific

237 leaf wetness sensors were placed, face-up, underneath a female and a male plant within the rhizosphere. Another leaf wetness sensor was placed an equal depth under a patch of bare ground. Temperature/ humidity/dewpoint sensors were also positioned within the canopy of a female and a male plant.

Light and soil-moisture data was recorded every 10 minutes using a CR1000 data-logger and a multiplexer. The means of data collected every 10 minutes were logged at a 30 minute interval. Light measurements were pooled into 4 groups; morning, late-morning, afternoon and evening. Due to the non-normality of the data set wilcoxon tests were used to compare groups between sexes. To test soil moisture differences statistically, voltage data was log-transformed and run through a GLM with "sensor" and "time" as interacting independent variables. Because sample size was low, (essentially a single representation of a felled female and a senesced male) care was taken to select a comparable male and female which clearly display the striking contrast in canopy pattern caused by significant culm-felling in females and senescence in males. Temperature/humidity and dewpoint were all compared between sexes using multiple t-tests.

Soil moisture sensors and PAR sensors were calibrated in the lab under controlled conditions revealing no significant differences between sensors. However soil moisture calibration involved rapid wetting and drying of sensors in a homogenous soil medium and may not account for the sensitivity of sensors to gradual and slight changes in soil moisture over time. It should also be noted that sensors appeared to be sensitive to slight changes is positioning so care was taken to reduce confounding variables when placing sensors.

Phenology

New culm growth was quantified through monthly measurements of newly emerging culms. The average height of seven emerging culms was recorded in each of twelve females and twelve males monthly. This was repeated throughout the year in order to track the new culm growth in males and females. Each month, individuals were selected randomly, thus including a larger sample of the entire population in the overall study.

To understand isotopic signals in plant tissue, the timing or sequence of tissue growth within a culm is important to consider because environmental factors can influence isotopic signals of plant tissue during growth leading to heterogeneity in the isotopic signal throughout the culm. Therefore culm elongation was recorded by comparing node lengths (one abscission scar/leaf sheath to the next) of emerging culms (not fully elongated) to the node lengths of mature culms. This showed the pattern of node elongation from the bottom to the top of culms during development to maturity.

RESULTS

Isotopes

Culm δ^{13}C signals were significantly affected by sex while culm-region and the interaction term did not have a statistically significant effect, F (3,44) = 4.77, p<0.01 (Table 1). Females had less enriched (more negative) δ^{13}C signals relative to males. Culm-region did not appear to have a predictable affect on δ^{13}C signals although the lowest values were seen in the lower-region of female culms (Figure 1).

Table 1: Results of a GLM testing δ^{13}C values against explanatory variables, 'sex' (Male or Female) and 'culm-region' (Bot or Top) as well as the interaction between the two. n =12.

Residuals:	Min	1Q	Median	3Q	Max
	-1.4555	-0.4066	0.1533	0.4689	0.9674
Coefficients:		Estimate	Std. Error	T value	Pr(>|t|)
	(Intercept)	-28.0942	0.1797	-156.310	< 2e-16 ***
	SexM	0.8991	0.2542	3.537	0.000967 ***
	RegionTop	0.4706	0.2542	1.852	0.070803 .
	SexM:RegionTop	-0.6360	0.3595	-1.769	0.083777 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.6226 on 44 degrees of freedom

Multiple R-squared: 0.2453, Adjusted R-squared: 0.1939

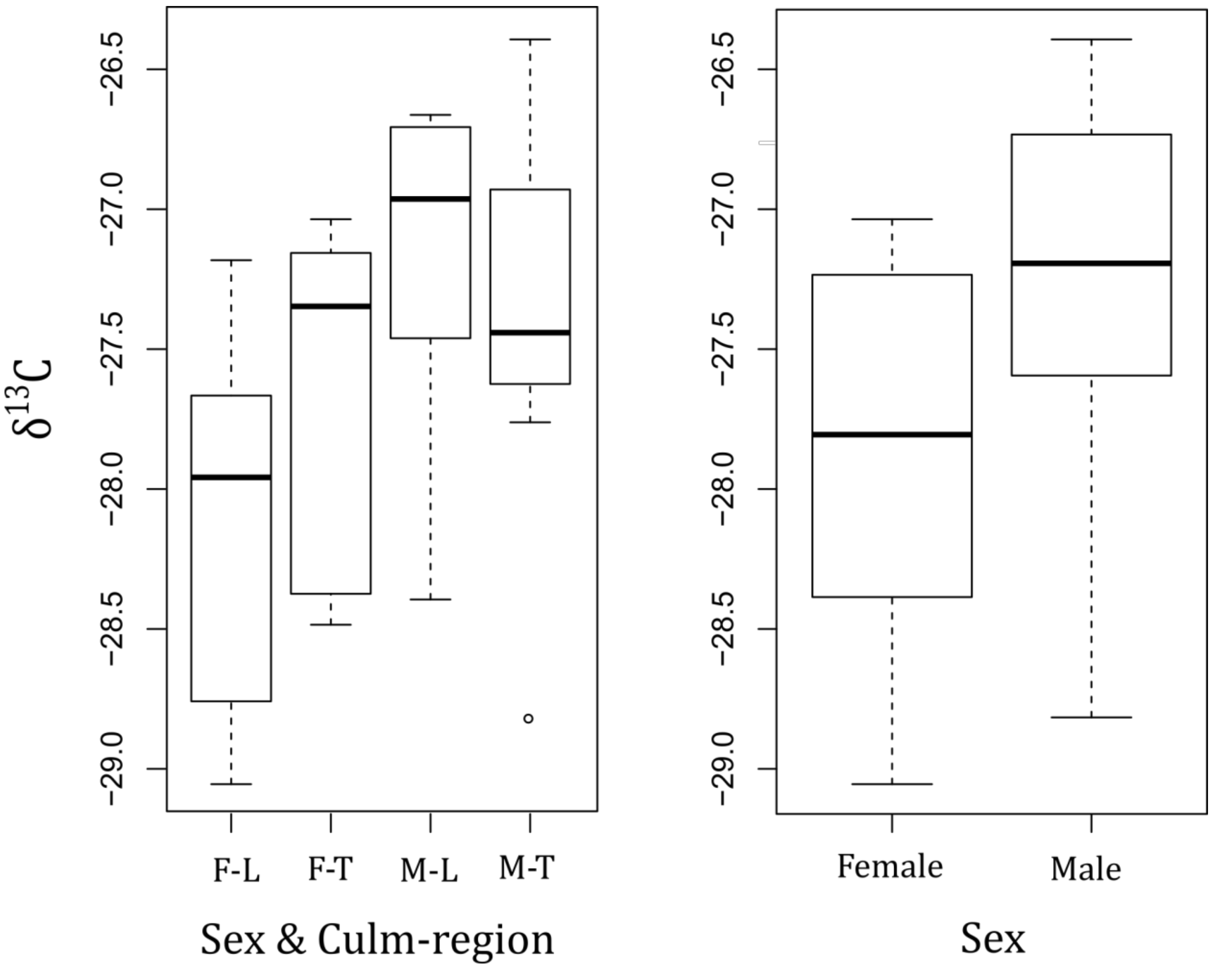

Figure 1: δ^{13}C signals in the living culm tissue of male (n=12) and female (n=12) *C. congesta*. Left: Comparisons between upper and lower culm-regions. Right: Combined, full-culm means. Box and whisker plot representing the median, inter-quartile range and min/max values.

Culm $\delta^{18}O$ signals were significantly affected by culm-region. Sex and the interaction between sex and culm-region did not have a significant effect, F(5,12) = 24.84, p<0.01 (Table 2). Culm $\delta^{18}O$ signals became more enriched on a gradient from the bottom to the top of culms (Figure 2).

Table 2: Results of a GLM testing $\delta^{18}O$ values against explanatory variables, ‘sex’ (M or F) and ‘culm-region’ (Bot, Mid, Top) as well as the interaction between the two. n =12.

Residuals:	Min	1Q	Median	3Q	Max
	-2.1399	-0.7830	0.2193	0.8932	2.0790
Coefficients:		Estimate	Std. Error	T value	Pr(>|t|)
	(Intercept):	31.7230	0.8347	38.007	7.08e-14 ***
	SexM	-0.1636	1.1804	-0.139	0.8921
	RegionMid	3.3179	1.1804	2.811	0.0157 *
	RegionTop	10.0890	1.1804	8.547	1.90e-06 ***
	SexM:RegionMid	0.1495	1.6693	0.090	0.9301
	SexM:RegionTop	-2.0265	1.6693	-1.214	0.2481
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 Residual standard error: 1.446 on 12 degrees of freedom Multiple R-squared: 0.9119, Adjusted R-squared: 0.8752					

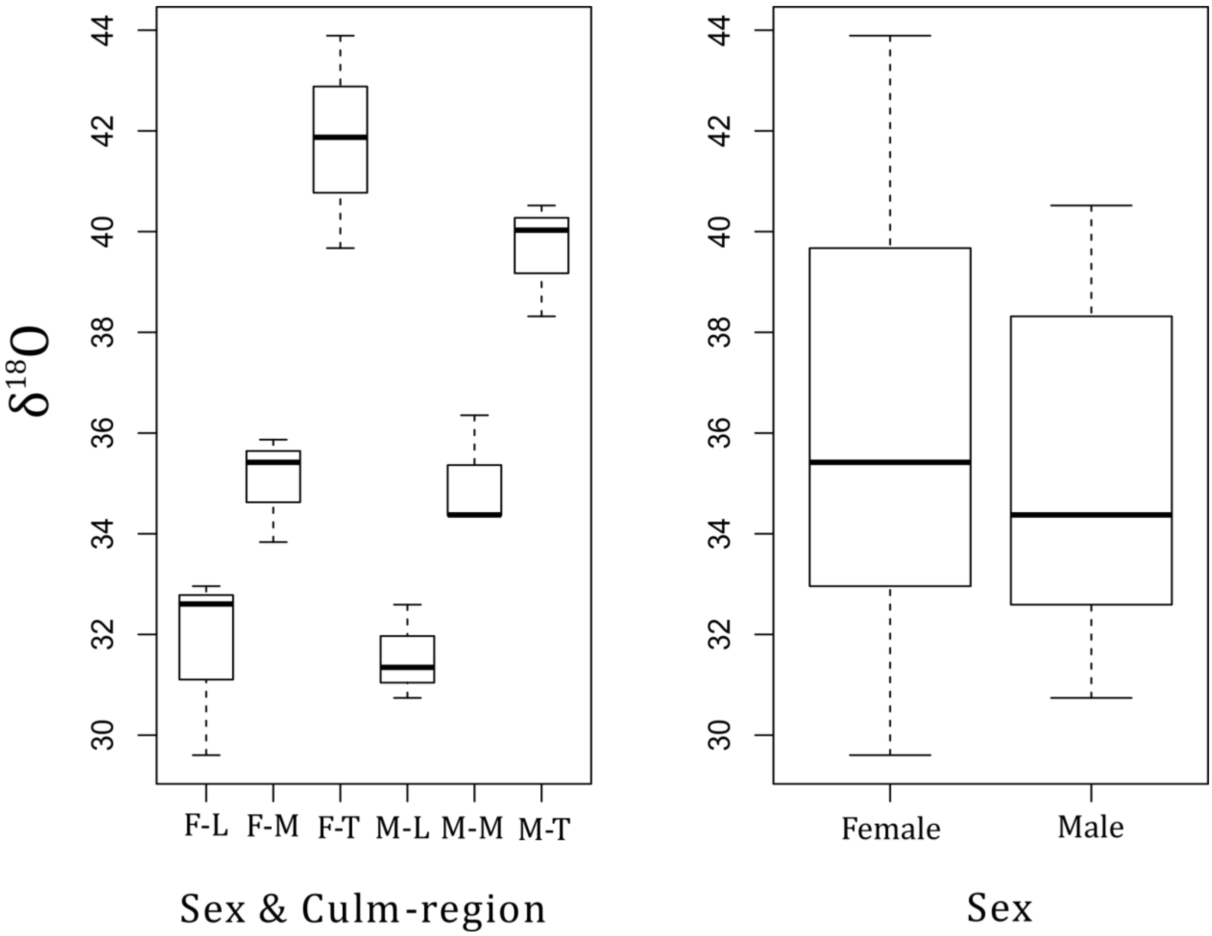

Figure 2: Holo-cellulose δ18O signals in the living culm tissue of male (n=3) and female (n=3) *C. congesta*. Left: Comparisons between lower, middle and top culm-regions. Right: Combined, full-culm means. Box and whisker plot representing the median, inter-quartile range and min/max values.

Gas Exchange

At all matching light levels (PPFD 50 – 1500 µmol/m^2/s), A, g & c_i means were not significantly different between males and females (Table 3) as indicated by a number of two-tailed t-tests ($p>0.05$ in all cases). Nett assimilation rate (A) appeared to respond most to changes in PAR between 0-500 µmol/m^2/s while higher light intensities were saturating in both sexes.

Table 3: A, g and c_i for male and female *C. congesta* at differing light intensities. Females n=2, males n=2. Mean ± (SE)

PPFD (µmol/m^2/s)	Females			Males		
	A (µmol.m^{-2}.s^{-1})	g (mol.m^{-2}.s^{-1})	c_i ppm	A (µmol.m^{-2}.s^{-1})	g (mol.m^{-2}.s^{-1})	c_i ppm
50	0.62 (0.17)	0.07 (0.01)	381 (3)	1.35 (1.18)	0.07 (0.01)	360 (31)
500	3.38 (1.75)	0.08 (0.02)	333 (20)	6.05 (0.20)	0.08 (0.01)	269 (9)
1000	3.74 (1.55)	0.08 (0.04)	320 (4)	8.74 (0.75)	0.09 (0.02)	224 (48)
1500	4.91 (2.38)	0.08 (0.03)	304 (20)	8.65 (1.70)	0.10 (0.03)	292 (9)

Additionally, modelled photosynthetic parameters showed no significant differences although sample size was small (Table 4)

Table 4: Photosynthetic parameters in *C. congesta* sexes extracted from modelled photosynthetic light response curves (female n=2, male n=2). Mean ± (SE)

	Female	Male	T-test p-value
Rd (-ve)	1.17 (0.09)	0.92 (0.47)	0.69
φ (Quantum Yield)	0.034 (0.001)	0.058 (0.031)	0.58
Amax	5.96 (2.20)	10.74 (1.69)	0.23
θ (Curvature Factor)	0.75 (0.25)	0.50 (0)	0.5
SSD (model strength)	2.01 (0.42)	2.7 (1.27)	0.69

Seasonal patterns of photosynthesis

Multiple t-tests showed no difference in A/g ratios between males and females in any months of the year ($p > 0.05$ in all cases) (2012-2014 Rob Skelton's data) and so male and female data was combined. This combined data can be used to predict seasonal WUE patterns (Figure 3) in both sexes and thus help to understand $\delta^{13}C$ differences found in this chapter. A/g was seen to be highest during the hottest, summer months and drop during winter months. (see Discussion for the influence of phenology and expected seasonal WUE on $\delta^{13}C$ in *C. congesta* sexes).

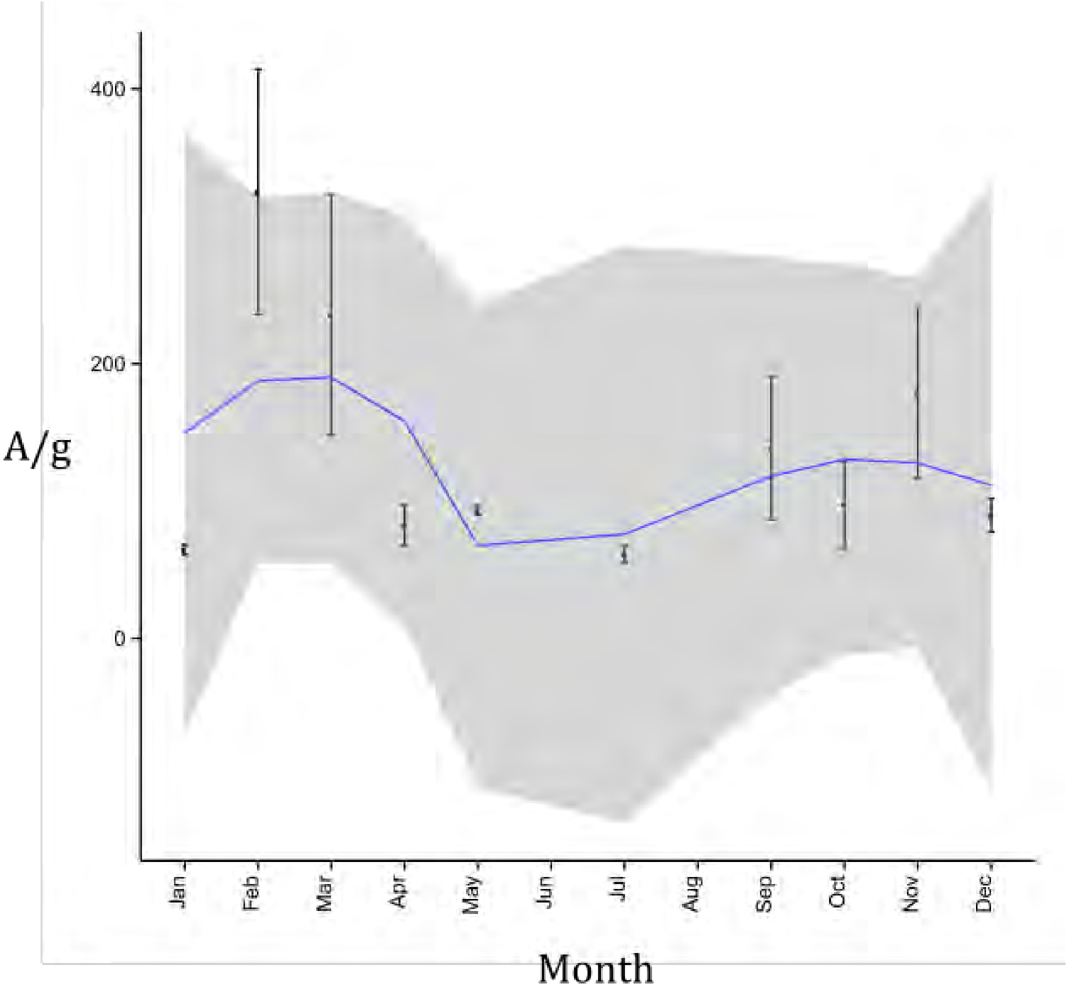

Figure 3: Mean general monthly intrinsic water-use efficiency patterns of *C. congesta* (both sexes, undifferentiated) from 2012-2014. Means ± SE ($5 < N < 25$). Solid, blue line represents a smoothed conditional trend with 95% CI shaded in grey.

Microclimate differences:

Mid-canopy (felling-level) interception of photosynthetically active radiation (PAR) (Figure 4) was consistently lower than expected full sunlight values (PPFD ±2000μmol/m^2/s) in both the male and female canopies. As expected, PPFD values increased from morning to afternoon, dropping again in the evening. The light penetration in the felled female canopy was only significantly higher during the peak afternoon hours while the males received significantly more light during the morning and late-morning (Figure 4 & Table 5). Light differences in the morning and late morning were small but due to a very large number of data points, differences were statistically significant (Table 5).

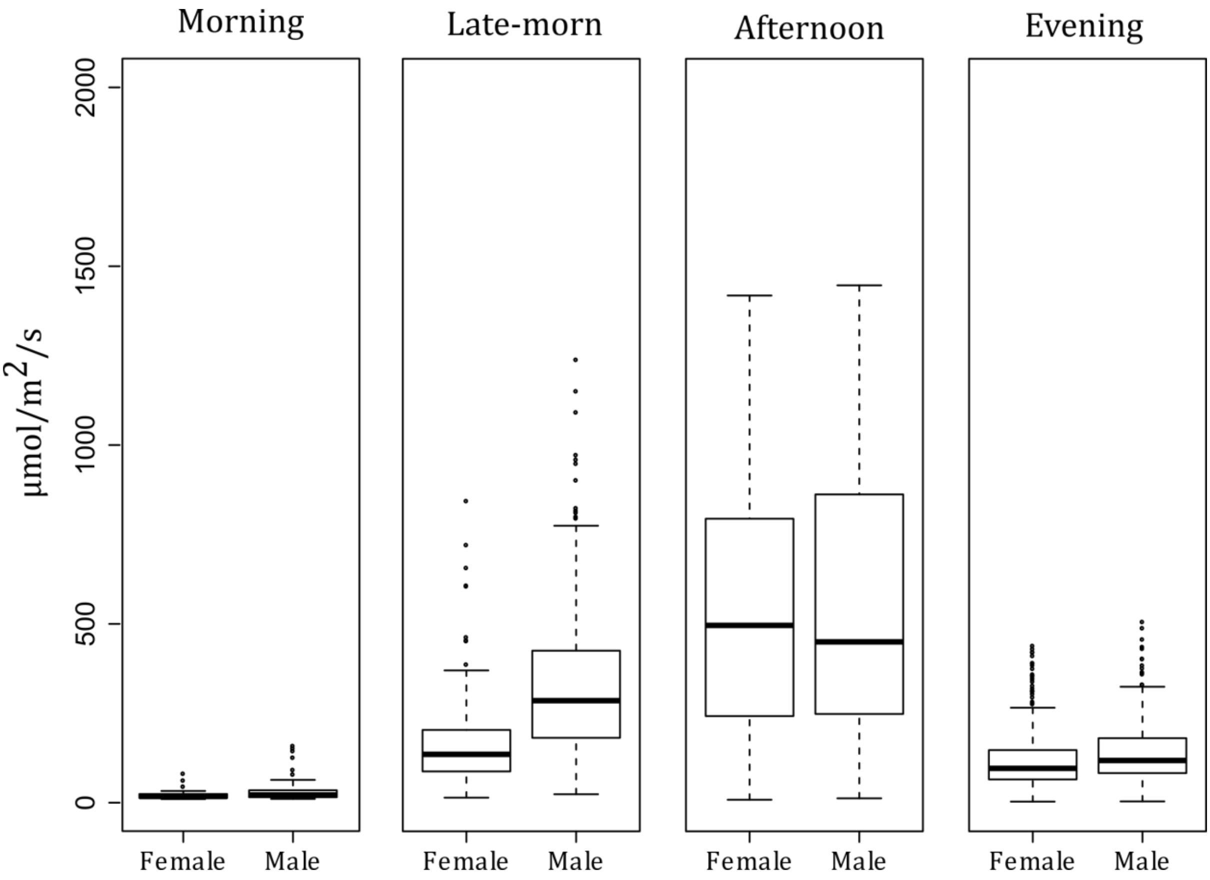

Figure 4: Comparison of Photosynthetic Photon Flux Density (PPFD) in the mid-canopy of a classically felled female and a non-felled male throughout the day. Morning (7-8am), Late-morning (10-11am), Afternoon (1-2pm), Evening (4-5pm). Box and whisker plot representing the median, inter-quartile range and min/max values.

Table 5: Statistical differences in the interception of photosynthetically active radiation (PAR) in the mid-canopy of a typical senescent male and a highly felled female *C. congesta* plant. Visual representation of data in Figure 4.

Time of day	Male vs. Female Wilcoxon W-stat	Summary	p-value
Morning	37531	M > F	**< 0.01**
Late-Morning	87524	M > F	**< 0.01**
Afternoon	138993.5	F > M	**< 0.01**
Evening	105457	F = M	0.28

Note: during gas exchange measurements, plants were observed to stop photosynthesising by midday, even on cool, overcast days. For this reason, only morning and late-morning values were considered as relevant for male/female photosynthetic comparisons.

In order to determine if the PAR sensor data was reliable, sensors placed in the example female and example male canopy were switched and left to collect data. The results showed that light interception throughout the day can be variable within the canopy and sensitive to slight changes in position because the patterns of light interception were not identical once sensors had been switched. The only consistencies were in the morning and late-morning where the example female canopy intercepted less light compared to males. This could be due to long morning shadows cast by surrounding vegetation affecting the selected female plant.

Soil moisture (Figure 5) was maintained at higher levels, to varying degrees, beneath both male and female *C. congesta* individuals during periods where bare ground dried up. Increased drying in soils around the male was seen during extended dry periods compared to the female which had relatively moist soils during these times. All three sensors were quickly saturated by rain events. All three sensors (bare ground, male & female) varied significantly differently from each other over time, $F(5, 41313) = 7072$, $p < 0.01$. Switching moisture sensors showed that mV readings were sensitive to changes in sensor positioning. For this reason only general wet/dry patterns were detectable and not detailed male-female moisture comparisons. A general pattern of soil drying in summer and soil saturation in winter was revealed by the three soil moisture sensors. Peak dry periods appeared to be in Jan – March while soils were saturated throughout the winter months, beginning in late April (Figure 5).

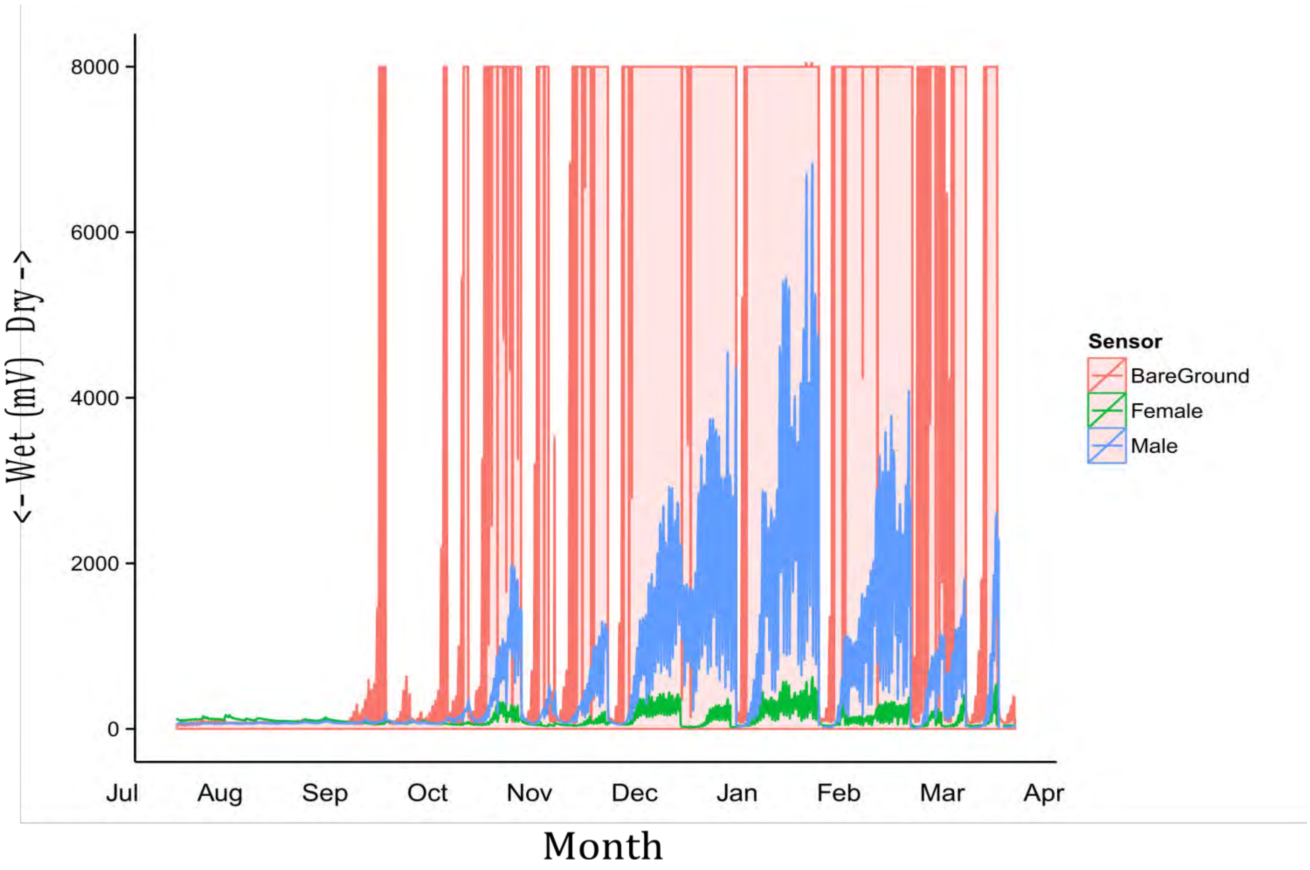

Figure 5: Comparison of voltage in leaf wetness sensors (proxy for soil moisture) in the soils beneath a senescent male, a highly felled female and in bare ground. Higher voltages indicate drying periods in the soil. Voltages tend to 7999 mV when no moisture is present.

Temperature, humidity and dew-point were very similar between sexes (Figure 6). Multiple t-tests (Table 6) revealed that male and female values were not significantly different throughout the day with the exception of the late-morning, where temperature, humidity and dew-point were all statistically different between males and females. This is likely to be related to the significant mid-morning PAR differences (Figure 4). However the effect size was very small and was not likely to have any physiological consequences (Figure 6). Exaggerated p-values can occur due to the very large number of data points (Sullivan & Feinn 2012).

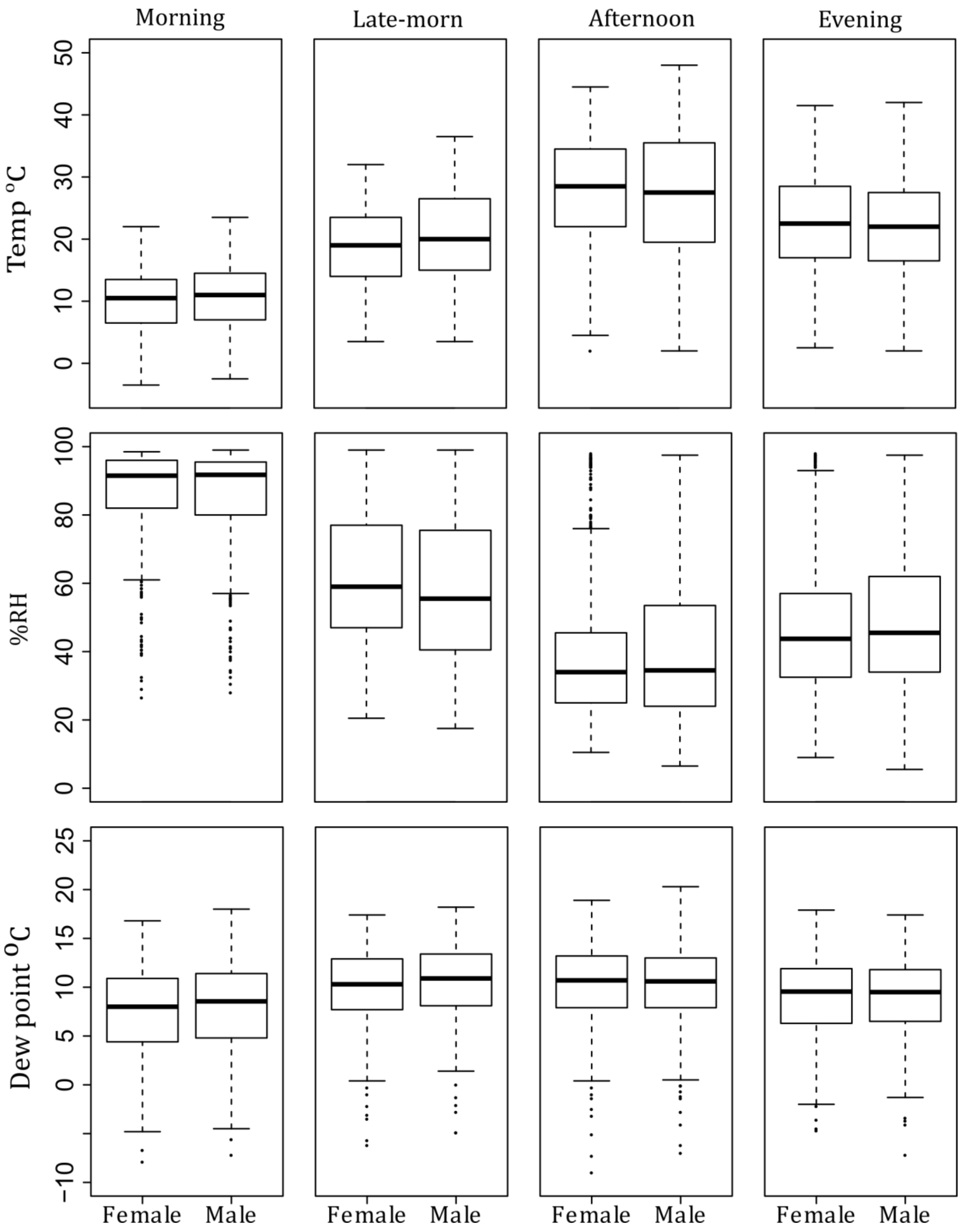

Figure 6: Mid-canopy dew point means at different times of the day throughout the year. Box and whisker plot representing the median, inter-quartile range and min/max values.

Table 6: Statistical differences in Temperature, Humidity and Dew Point between the sexes of *C. congesta*.

	Temperature		Humidity		Dew Point	
Male vs. Female	T-stat	p-value	T-stat	p-value	T-stat	p-value
Morning	-1.81	0.07	-1.03	0.30	1.41	0.16
Late-morning	-3.91	**≤0.01**	2.21	**0.03**	2.42	**0.02**
Afternoon	0.030	0.98	-0.72	0.47	-0.01	1
Evening	1.36	0.17	-1.61	0.11	-0.32	0.75

C. congesta phenology

Timing of new culm growth

Extended linear trends with 95% CI suggest that the growth of new culms began asynchronously between sexes (Figure 7). This was confirmed by early observations made before new culm growth measurements began in May 2014. Male growth began in Feb and female growth began in April. Both flowered synchronously in September. The height of female new growth was consistently lower compared to males throughout the pre-flowering growth period, due to the head-start in males. However, faster elongation of new female culms allowed new female culms to catch up to males in height by the time of flowering in September. After flowering, culm elongation continued synchronously in sexes for another few months until reaching full length.

Flower development

In males, new culms began to produce inflorescences during late July with the heads of new male culms swelling terminally. In August, males produced inflorescences made up of many small spikelets. Anthers emerged from spikelets in September. Females showed signs of pistil development in August. Peak flowering occurred synchronous to male flowering in September, with small, white, fluffy stigma protruding from the tips of female culms (Figure 7).

Culm elongation patterns

Culms were observed to undertake intercalary growth. New culms emerging from the rhizomes were purple/brown and encased in hard protected sheaths. Nodes were condensed and not separated by green internodes. Elongation of internodes began at the base of new developing culms in males and females because internode lengths were significantly longer closer to the base at this stage. In mature culms, internodes were roughly equal in length along the culm (Figure 8).

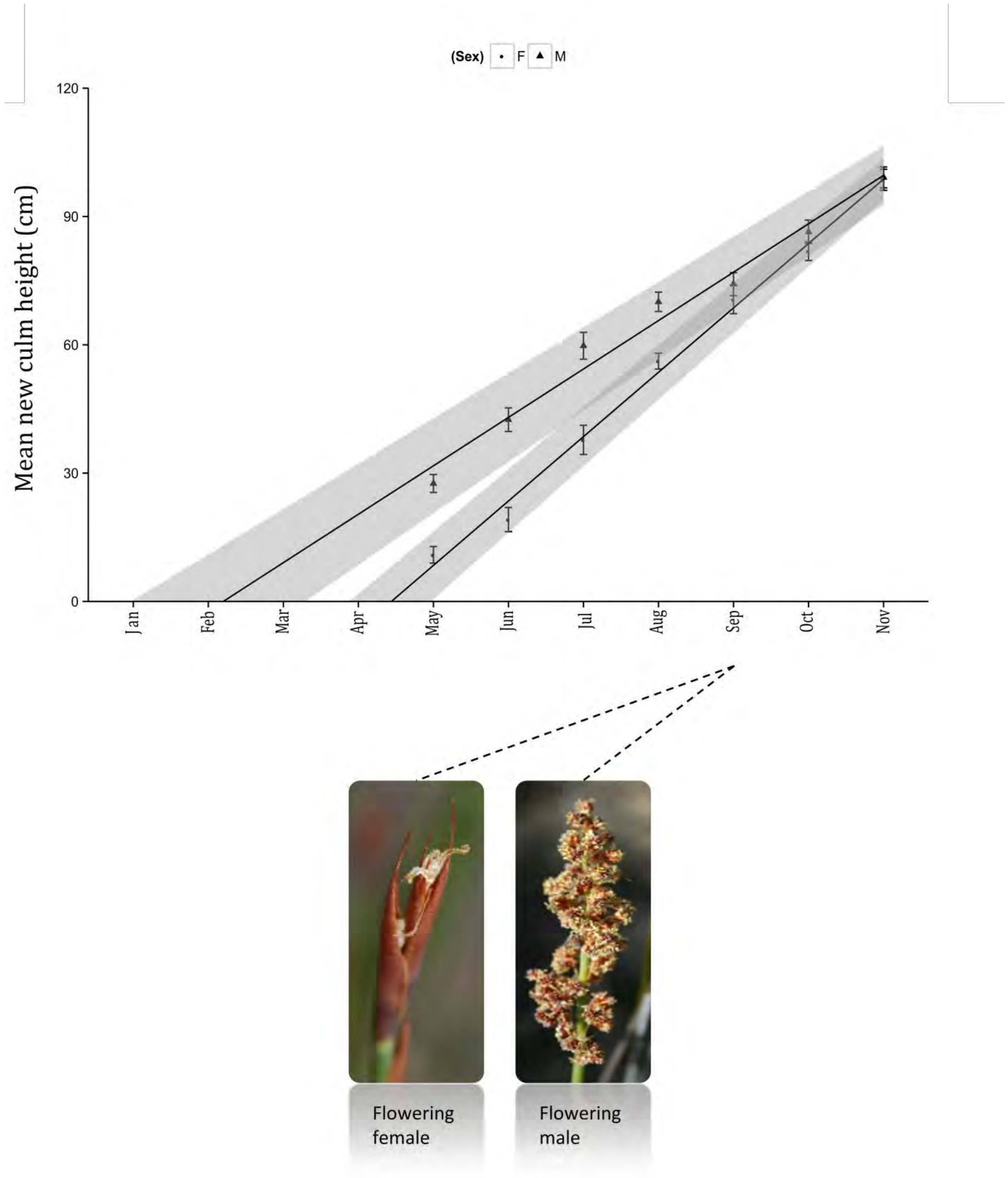

Figure 7: Culm growth of male and female *C. congesta* plants in 2014. Mean ± (SE). Linear trend (black line) with 95% CI (shaded) fitted and extended to y=0. Timing of flowering displayed.

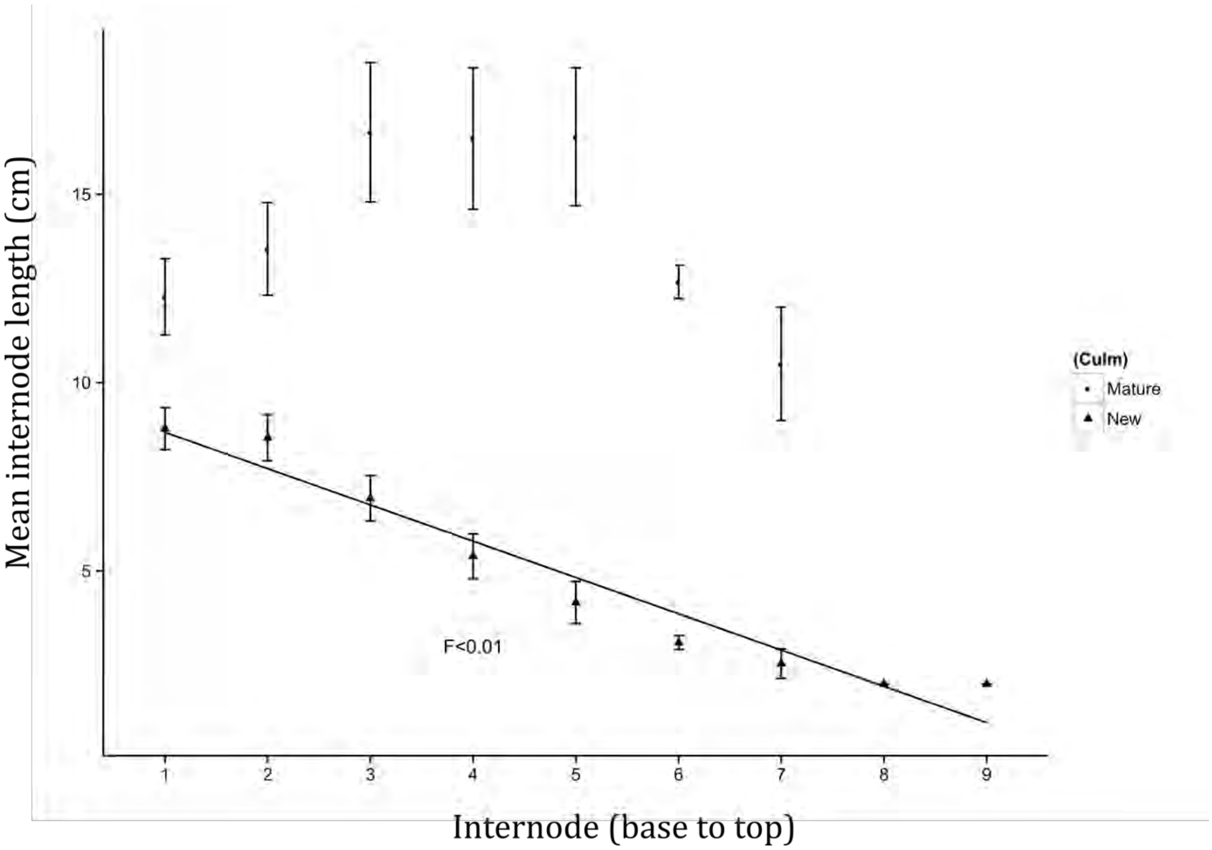

Figure 8*:* Elongation of new culms in 2014. Internode lengths from base to tip in culms. Mean ± (SE). A significant linear trend is fitted through the elongation of developing 'new' culms.

Seed development

Females began to produce seeds shortly after flowering in September. Inflorescences gradually swelled until seed-fall a full year later (October – December). During this time, post-flowering male culms remained bare (Figure 9). I therefore assumed that female reproductive effort is significantly larger than males overall, especially in the period between flowering and seed fall. Initial male investment into flowering (around September) was likely to be higher than in females however this period was short-lived in comparison to seed production in females.

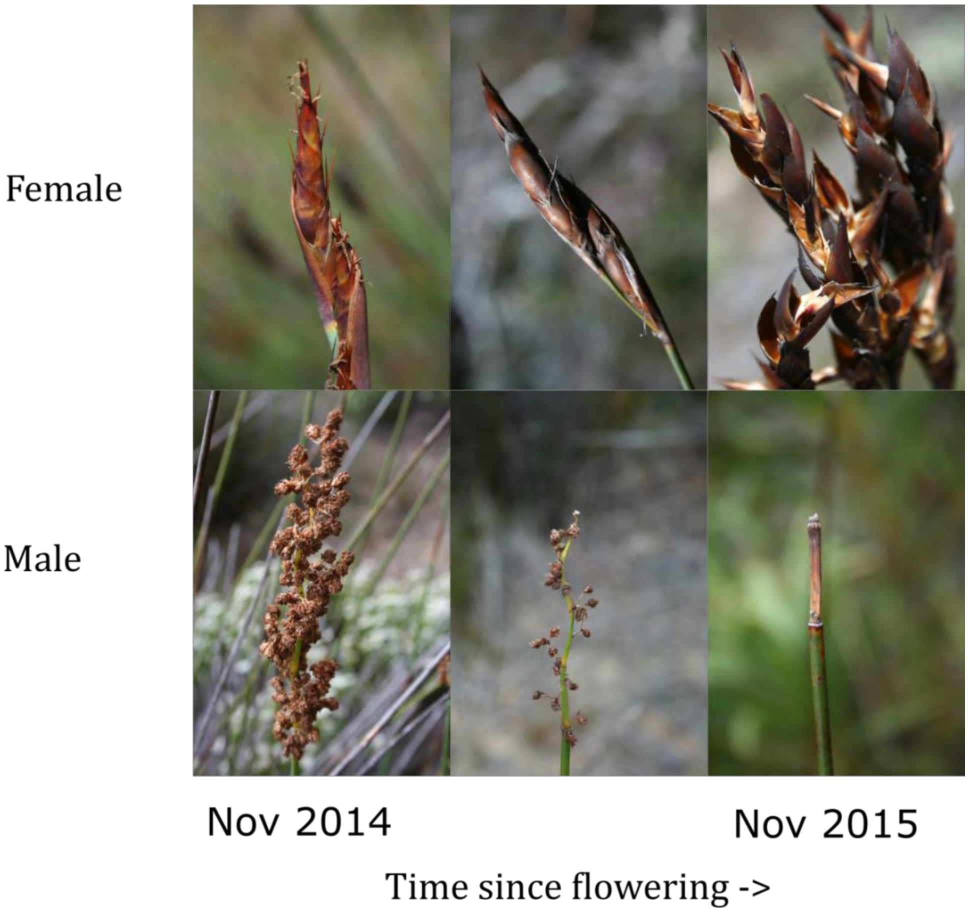

Figure 9: Visual representation of developing seeds in females and the equivalent culm stages in post-reproductive males.

DISCUSSION

Comparing photosynthetic performance in *C. congesta* sexes

Gas exchange measurements taken on mature culms in 2015 as well as data from 2012-2014 (Skelton 2014) revealed that photosynthetic performance in terms of the intrinsic WUE (A/g) was not different between *C. congesta* sexes during any monthly measurements. However A/g followed a seasonal pattern in both sexes, increasing in the hottest months of the year (Jan, Feb, March) and decreasing in the wet, winter months (June, July) (Figure 3). Culm $\delta^{13}C$ signals should reflect the long term intrinsic WUE (A/g) of sexes because the ratio of A/g during photosynthesis directly influences c_i and thus the $\delta^{13}C$ signals stored in culm carbon (Farquhar 1983 *et al*; Farquhar *et al* 1989). Therefore because A/g data lead to the expectation of no sex related intrinsic WUE differences, another explanation for the significant (yet small) sexual differences in mature culm $\delta^{13}C$ signals is required. Females had lower (more negative) overall average culm $\delta^{13}C$ signals indicating an overall increase in c_i relative to males (Farquhar *et al* 1983; Farquhar *et al* 1989). While statistically significant, the % effect size of difference in $\delta^{13}C$ signals between sexes was unlikely to carry much physiological significance for plant fitness with differences between sexes being much lower than those found between dioecious species which had experienced habitat specialisation between sexes (Dawson & Bliss 1989; Dawson & Ehleringer 1993). Regardless of the size of the $\delta^{13}C$ difference, significant differences in $\delta^{13}C$ signals between sexes are likely to be reflective of other significant differences which may be more important to understand.

The lack of sex related difference in A/g over time (2012 – 2014 & 2015) in mature culms suggests that $\delta^{13}C$ difference must have been accumulated in culm-tissue before culm maturity (A/g in emerging culms were not measured). I propose that phenological differences in the timing of culm elongation (Figure 7) are the most likely explanation sex-specific $\delta^{13}C$ signals because patterns of $\delta^{13}C$ in males and females can be explained by the differing environmental conditions and expected intrinsic WUE (Figure 3) experienced by emerging culms of each sex (Figure 7). Specifically, increased overall moisture and decreased light was experienced by emerging female culms because they grew later (closer to winter), and more rapidly than males. New culm growth in males incorporated more high light, low moisture periods into growth either side of the wet winter. This would have shifted female $\delta^{13}C$ signals to become more negative relative to males (Farquhar *et al* 1989; Retuerto *et al* 2000), agreeing with the pattern seen in mature culms. In young, developing culms, $\delta^{13}C$ signals were presumably less similar between sexes than in mature culms (*not tested) however the detectability of this difference was likely to be reduced over time after female and male new culm growth reached the same height and continued to grow in synchrony after flowering adding more carbon to culm tissues. The patterns of intercalary, bottom-to-top culm node elongation could support this hypothesis because in this context it can explain the apparent patterns in $\delta^{13}C$ between upper and lower culms of both sexes. Female growth which began in the wettest, lowest-light part of the year would be expected to have to lowest $\delta^{13}C$ values in the lower region of the culm which grew during this time. Males, starting culm growth in the hottest, highest-light months in the year would be expected to have the highest $\delta^{13}C$ values in the lower part of the culm. This pattern is seen in $\delta^{13}C$ results (Figure 1). Lower culm region growth of some males would also coincide with some saturated winter months which is probably the cause of minimum male lower culm $\delta^{13}C$ values being low (within the range of lower culm female values). The $\delta^{13}C$ values in the upper culm region of both sexes were similar. This is presumably due to the seemingly differing rates of pre-flower culm growth leading to simultaneous growth of the upper culm region coinciding with the same environmental light and moisture conditions.

Photosynthetic parameters (Amax, Rd, Quantum Yield, Curvature factor) were also not statistically different between *C. congesta* sexes, mirroring the lack of difference in the raw gas exchange data (A, g, ci). This could be the result of sexes having non-different photosynthetic machinery and similar constraints in terms of adapting complex, interlinked, photosynthetic processes to differing sexual requirements. The similarity in culm nitrogen concentration between sexes supports this view because photosynthetic machinery is composed largely of nitrogen (Evans 1989).

Average $\delta^{18}O$ values were not significantly different between sexes. Because $\delta^{18}O$ signals represent the integrated water status of plants during growth, similar values between sexes suggests that $\delta^{13}C$ differences (which were probably formed during early culm growth – see above) are more likely to be related to an assimilation response rather than differences in water-status and stomatal conductance (Scheidegger *et al* 2000). Mature culm $\delta^{18}O$ values displayed increasing enrichment along culms from bottom to top (probably due to increasing transpiration along the culm) but this pattern did not appear to influence $\delta^{13}C$ values.

Photosynthetic differences (point-in-time & integrated) have often been related to differences in reproductive allocation (seed or pollen production) between dioecious males and females (Alvarez-Cansino *et al* 2010; Dawson & Ehleringer 1993; Dawson & Bliss 1989; Wheelwright & Logan 2004). The lack of functional differences in mature culm photosynthesis between *C. congesta* sexes suggests that females did not adjust photosynthetic performance in response to their greater reproductive costs. This is not surprising however because habitat partitioning, natural dimorphism, nutrient concentration differences or overall reductions in plant fitness did not appear to occur (pers. obs.). Female plants appeared to maintain a similar photosynthetic performance to males throughout the year, regardless of greater reproductive effort, nutrient losses and changes in canopy structure due to culm-felling.

The influence of micro-climate (affected by culm-felling) on photosynthesis

Despite major differences in the architecture of heavily felled females plants and highly senesced (un-felled) male plants, there were no major consistent differences in mid canopy light (PAR) interception. This could be expected from restio canopies which are generally open to light interception and do not appear to be highly affected by physical changes to the canopy structure. Varied results in mid-canopy PAR data verify this showing that culm-felling did not have a predictable effect on light interception. Without major differences in the interception of photosynthetically active radiation, it stands to reason that culm isotopic differences were not caused by maintained differences in light due to architectural differences, especially seeing as δ^{13}C signals were more negative in females, opposite of what would be expected from the higher light interception that was hypothesised for felled females.

Switching PAR sensors revealed that small sensor surfaces were presumably sensitive to small changes in position within the restio canopies. Therefore even though time was taken to select a classic example of a felled female canopy versus a senescent male canopy, small changes in shading due to positioning within each canopy made it hard to draw strong conclusions about the overall mid-canopy light interception in male versus female *C. congesta* plants.

Soil moisture data showed that the male canopy dried up more than the female canopy. While a mechanism leading to this pattern is not clear, this pattern could potentially provide another explanation for lower δ^{13}C (WUE) values in females. Females with a larger total number of culms (stumps or full) may intercept more moisture (rain/fog – common at Jonaskop) than males. Switching sensors suggested that moisture data was also sensitive to slight changes in positioning. To confirm that differences in the magnitude of drying between the male and female sensors were due to the actual moisture differences or due to differences in the sensors' sensitivity to moisture/positioning, a larger sample of the population would have to be tested in future (see Methods for calibration details).

Temperature, dew point and humidity did not appear to be influenced significantly by microclimate differences between typical senescing-male – felled-female canopies.

Synthesis:

This section includes results from previous chapters because they are required in order to understand the significance of the results reported in this chapter. The lack of male/female differences in photosynthetic performance, nutrient resorption (Chapter 3) and habitat partitioning (pers. obs.), suggested that little physiological compensation or specialisation occurred in females in response to their higher reproductive costs and nutrient losses (Chapter 3: Table 7). There was also no evidence of reductions in plant growth, reproduction or photosynthetic capacity (A_{max}). Females actually grew larger than males overall (Chapter 2: Table 2) and still produced seeds on every new reproductive culm (pers obs.). Growing a larger number of overall culms could potentially compensate for the loss of culm tissue in females due to *Rhabdomys* however the mechanism behind this achievement is not yet known. The role of phenological plasticity in allowing flexibility between sexes is probably important in order to understand how males and females with differing pressures/requirements manage to coexist (Araya *et al* 2010). Phenological flexibility presumably allowed males and females to use the resources available to them most efficiently because adjustments in the timing of growth and other important processes can reduce the impact of overlapping internal and external resource pressures (Delph 1990; Milla *et al* 2006). The capacity of females to continue with high levels of growth (higher than in males) and reproduction could also be explained the translocation of stored nutrients in rhizomes (no data). The translocation of nutrients between below and above ground organs has been shown to be important as a strategy against nutrient limitation in some graminoids (Hopkinson & Schubauer 1984). Furthermore it has been shown that due to the existence of underground storage organs, graminoids have a unique ability to respond to high levels of herbivory with compensatory growth (Bryant *et al* 1983). Females could have translocated rhizome-stored nutrients to maintain short-term growth and reproductive fitness, while increasing long-term vulnerability to periods of high environmental stress.

CHAPTER 5

General Conclusion

This study is among the first to show that sex-specific plant-animal interactions in dioecious plant species can lead to noticeable trait differences between plant sexes that are not necessarily adaptations or tradeoffs related to different reproductive requirements (Darwin 1877; Lloyd & Webb 1977; Antos & Allen 1990; Dawson & Ehleringer 1993; Delph 1999; Obeso 2002; Harris & Pannell 2010). I documented a previously undocumented sex-specific plant-animal interaction where the nut-like seeds of female restios (*C. congesta*) attract physically destructive rodent predators (*Rhabdomys)*, leading to noticeable male-female plant canopy differences. I also succeeded in investigating a number of plant sexual differences in *C. congesta* which could be linked to this interaction. In the case of *C. congesta* the evidence suggests that the loss of pre-senescent culm tissue from females significantly affects canopy architecture (chapter 2) and may lead to losses in nutrient availability (chapter 3).

This study showed that *C. congesta* male and female physiology was highly similar regardless of their differing reproductive efforts, sex-specific nutrient losses and canopy architecture. Females were even able to grow larger than males. This indicated that *C. congesta* plants were very efficient at conserving nutrients and maintaining physiological functionality, regardless of the different sex-specific circumstances. This was potentially achieved through the use of compensatory mechanisms such as phenological changes which can mask the effects of differing sexual pressures and trade-offs and allow plants to best use the resources around them. Another possibility is that rhizome-stored nutrients allowed females to undertake compensatory growth. This could increase vulnerability to nutrient losses, and future periods of resource limitation.

Because the entire Restionaceae family is dioecious, and because the destructive seed acquisition by rodents through culm-felling could occur commonly in the family, it is important to understand this process so that when present, we can include it in future studies of dioecy in Restionaceae, which are lacking in the literature. Beyond the Restionaceae family, any sex-specific interaction in deioecious species which has a direct, physical effect on either plant sex could lead to a variety of trait differences between sexes. Physiological comparisons between dioecious species which do not take such sex-specific interactions into account might misinterpret physiological differences (if found), falsely relating them to differences in reproductive requirement, when in fact the differences were ramifications of the sex-specific interaction.

Not related to the study of dioecism, but still equally relevant is the effect of pre-fall seed predation on the success of myrmecochorous seed dispersal. This would be important to quantify further in future studies seeing that studies of Restionaceae contribute significantly to seed dispersal theory (Bond & Slingsby 1983; Meney *et al* 1994; Caddick & Linder 2002; Newton *et al* 2006; Lengyel *et al* 2010). While rodent, seed predation has been commonly investigated, most studies focus on seeds which have fallen to ground. In the case of culm-felling in *C. congesta*, seeds are eaten by *Rhabdomys* before reaching the ground thus excluding ants from interacting with these seeds and presumably reducing myrmecochorous seed dispersal success.

In conclusion our study introduces a new twist to the well established study of dioecism in plants. Sex-specific plant-animal interactions could provide interesting information about the flexibility and sensitivity of dioecious plants to changes in their form and function.

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