Comparing investment in reproductive tissues and new leaf shows plant growth is more determinate than we thought

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# Abstract

Intro needs to narrow more towards the end

Focus on question of how determinate or not plant growth is

* hypothesis that is consider replacement, more determinate than we think [[This does suggest a comparison figure whereas I argued to take that out. Hmm. Maybe putting in Suppmatt with quoting max RA in text is sufficient. Or table?]]
* Focus on productive part of plant - -is it growing? Need to consider replacement.

# Introduction

A fundamental trade-off faced by all plants is how to partition available energy into growth versus reproduction (Obeso 2002; Wright *et al.* 2005; Weiner *et al.* 2009; Wenk & Falster 2015). Greater investment in growth translates to more rapid height increases and/or greater leaf area, resulting in greater access to light and higher photosynthetic yield, in turn leading to improved competitive outcomes and consequently higher survival (Wright *et al.* 2010). In contrast, reproductive production directly and immediately increases fitness through seed production, but potentially at the expense of future reproductive value. Quantifying the lifetime pattern of a plant’s investment into vegetative versus reproductive tissues is a prerequisite to diverse research questions including constructing life history tables, parameterizing growth models, modelling global energy sinks, and describing what tissue types are present to con

The growth-reproduction trade-off is most frequently quantified as reproductive allocation (RA), the proportion of surplus energy that is invested in reproduction (versus growth, storage, or defence) in a given year (Thornley 1972; de Wit 1978; Kozlowski 1992; Mäkelä 1997). *Surplus energy* is defined as the energy remaining after so-called maintenance costs: the energy needed to replace shed vegetative tissues.

A lifetime plot of RA, termed an RA schedule, summarizes shifts in investment into growth versus reproduction as a species grows and ages, reflecting how the outcome of the trade-off shifts with plant size or age. Early theoretical explorations of this trade-off suggested an individual’s fitness was always maximized by first investing solely in growth and then ceasing growth and having a single year of reproduction followed by death (Cole 1954), termed the big-bang strategy. However, perennial plants, excepting a small number of specialized species (Young 2010; Thomas 2011), exhibit a more nuanced transition of resources from growth to reproduction and have many years of simultaneous growth and reproduction, termed an iteroparous life history strategy. Moreover, they are considered to display indeterminate growth, continuing to increase in size until death. Such species’ RA schedules are termed “graded”.

Both modelled and the few available empirical RA schedules vary notably in shape across species, with some species displaying a distinct asymptotic maximum RA value, others continuing to increase gradually in RA until death, and a few displaying declining RA schedules, with a decrease in RA among the oldest plants (reviewed in Wenk & Falster 2015). In contrast to these graded RA schedules, big-bang species display a sudden year-upon-year transition from investing all surplus energy in growth to reproduction, immediately halting all vegetative growth and followed by death within months to a few years. For all curves, as RA approaches 1, the plant is, by definition, no longer investing any proportion of its surplus energy in growth and therefore ceases to increase in size. The plant should be investing sufficient energy in vegetative tissues to maintain its current stature and leaf area, but not to increase them.

Optimal energy models have shown that RA is a sound way to illustrate the growth-reproduction trade-off. They have explored what factors lead to various graded RA schedules, showing that a gradual shift in resources from growth to reproduction is expected if environmental conditions are stochastic (King & Roughgarden 1982) or if the outcome of the growth-reproduction trade-off shifts with a factor that changes across individuals of different age, size, or other variable. Of particular note, if mortality declines with size or age, as is observed for many perennial plants across much of their lifespans (###), it is optimal for individuals to invest more modestly in both growth and reproduction across multiple years (Charnov and Schaffer 1973; Reznick and Endler 1982; Engen and Saether 1994). Declining photosynthetic rates with plant age (Iwasa & Cohen 1989), not being deciduous (Pugliese & Kozlowski 1990) and relative declines in seed production at higher rates of reproductive investment (Miller, Tenhumberg & Louda 2008) have also been modelled as factors leading to simultaneous investment in growth and reproduction across multiple growing seasons. These theoretical explorations indicate that the shape of the RA schedule, age at reproductive maturity and the maximum RA achieved reflect species’ life history strategies (e.g. mortality, seed production) and key functional trait values (e.g. leaf lifespan, photosynthetic capacity, height, seed size)

Despite the obvious importance of tabulating investment patterns across an individual’s lifetime and across species with different life history strategies and functional traits, there are a quite limited number of species for which we know the lifetime energy investment in reproductive versus vegetative tissues (reviewed in Weiner 2009, Wenk & Falster 2015) and no community-level comparative studies exist. Moreover, the studies that do exist differ in how energy allocation to vegetative and reproductive tissues is calculated, potentially leading to quite disparate values for tissue investment. Many studies use seed weight as a proxy for reproductive investment, likely to be a gross underestimate in long-lived perennial plants with low seedsets. As a result of the paucity of data it is difficult to identify if there are trends in how either absolute or relative investment to different tissue types shifts within individuals as they grow and age or across species with different functional traits or life history strategies.

RA=1 indicates that all surplus energy is being invested in reproduction, at the expense of any growth to increase size. Of key importance to understanding the diversity of RA schedules displayed by different species is assessing if and when in a plant’s lifespan RA=1 is reached and what follow-on effects this has on the plant’s growth and survival. Perennial, iteroparous plants are generally presumed to continue growing throughout their life, with “growth” suggesting increased leaf area, stem mass, and height. Two questions arise from this assumption, whether production is truly increasing throughout a plant’s life and how much of this energy production goes to surplus energy in older, larger plants.

First, despite the general assumption that in long lived plants, total energy production is maintained or increased across time, a collection of evidence that suggests some older and taller plants may actually decrease their total energy production due to factors including declining leaf area (Ryan, Binkley & Fownes 1997; Kashian, Turner & Romme 2005; Nock, Caspersen & Thomas 2008) (Bond-Lamberty et al. 2002, Leuschner et al. 2006), decreasing photosynthetic capacity (Ryan *et al.* 1997; Day, Greenwood & White 2001; Niinemets 2002; Thomas 2010), increasing hydraulic limitations (###), crown abrasion (Ryan *et al.* 1997), and declining nutrient supply (###). These changes would lead to the observation that GPP can asymptote as plants reach middle age and decline in older stands (Gower, McMurtrie & Murty 1996; Ryan *et al.* 1997; Drake *et al.* 2011; Tang *et al.* 2014). These data suggest that many perennial species will show a gradual decline in the pool of energy available to grow to a bigger size*, before* considering that a proportion of surplus energy is being siphoned to reproduction. This is especially relevant given a number of optimal energy models that suggest the growth-vegetation trade-off is influenced by energy availability (Iwasa & Cohen 1989; Reekie, Budge & Baltzer 2002; Katsukawa, Katsukawa & Matsuda 2002). Determining changes in total energy production with shifts in plant size and age is therefore part of understanding observed RA schedules.

Second, as plants get larger and develop a larger leaf canopy, the energy that must be invested in maintaining their leaf canopy increases. If the rate at which energy is produced begins to slow and leaf replacement costs increase, surplus energy may plateau relative to plant size (or another plant production metric). Simultaneously, RA is likely to increase due to a shift towards reproductive investment (in the growth-reproduction trade-off) in older plants. In combination, it is possible that small declines in surplus energy availability in middle-aged to older plants, in conjunction with increased RA, might mean that reproductive investment rapidly consumes much of the pool of surplus energy explaining. This lack of surplus energy would feedback to put a rapid end to increases in plant height and leaf area – or worse. Indeed, Genet (Genet, Bréda & Dufrêne 2010) observed that the age-related declines in growth were not fully offset by increased reproduction, such that the decline in growth was greater than the increase in reproduction.

And both its leaf area and RA increase, there is a rapid increase in the pool of energy invested in reproduction and a declining rate of increase in the pool of energy invested in growth to increase size, such that plant growth rapidly asymptotes with the onset of reproduction (###Thomas).

The lack of studies that have carefully tabulated energy investment to all reproductive tissues, vegetative growth to increase size, and vegetative tissue replacement across multiple ages, means we have a poor understanding of how the interplay between energy investment to these three sinks are plants grow and age. Moreover, we lack community level comparisons to understand how species with different life history traits should display different RA schedules. Surprisingly, increased relative investment in reproduction (increased RA) is generally omitted as an explanation for declines in leaf area with increasing plant age. Many ignore any mention of reproductive investment (###Drake) and while others indicate that a shift of resources to reproduction with age is a hypothesis which needs more data (Ryan *et al.* 1997; Becker, Meinzer & Wullschleger 2000; Day *et al.* 2001; Tang *et al.* 2014)

Determining how species differ in their energy investment to three key tissue pools, vegetative replacement costs, vegetative growth to expand size, and reproductive investment, and how these patterns shift with plant size and age, is necessary to understanding why specific RA schedules are observed. These data will also yield insight to how vegetative replacement costs influence the pool of surplus energy to be divided between vegetative growth to expand size and reproduction and how this affects the resultant RA schedules. In particular, we explore:

1. How important it is to account for all pools of reproductive investment in assessing reproductive investment.
2. Whether species with different RA schedules differ in key life history traits, including lifespan, height, and age at first reproduction. We predict that species aligning themselves with the end of the life history continuum that are shorter lived, have a shorter stature, and begin reproducing at a younger age will also reach a higher maximum RA and reach a maximum RA earlier in their lifespan.
3. How proportional energy investment into vegetative tissue replacement, vegetative tissue growth, and reproductive tissue production shifts as a species ages. We predict that RA increases throughout life, approaching RA=1 toward the end of life, thereby impacting growth to increase size.
4. Whether in any species RA approaches or reaches 1 well before the end-of-life, indicating some perennial species are more determinate growers than generally assumed.

To address these questions we conducted a study in coastal heathland in eastern Australia, quantifying RA and vegetative maintenance costs for 14 dominant perennial species at 6 ages. These species differ for a collection of key life history and functional traits, including lifespan, maximum height, specific leaf area, wood density, and leaf nitrogen content. These species are all known to be iteroparous.

# Materials and methods

## Study system

The study was carried out in Kuring’gai National Park, northeast of Sydney, Australia. The sandstone surfaces throughout the park host a fire-driven coast heath community (Kodela & Dodson 1988). Fire regimes under traditional aboriginal management are unknown, but current New South Wales National Parks and Wildlife Service (NSW NPWS) management practises seek to achieve an average interval between 7-30 years to maintain the current floristic diversity (NSW Office of the Environment 2006). The community includes perennial species that re-sprout following fire and also obligate seeders, species that are killed by fire and re-establish from seed. The obligate seeders included in this study germinate within a year of the fire and often after the next rain. Since the fire history of the park is well documented, the age of obligate seeders at a site can be estimated. In total, we selected 14 obligate-seeder, woody perennials that are common in the community, with asymptotic heights ranging from 0.5 m – 5 m. They were *Banksia ericifolia* (Proteaceae), *Boronia ledifolia* (Rutaceae), *Conospermum ericifolium* (Proteaceae), *Epacris microphylla* (Ericaceae), *Grevillea buxifolia* (Proteaceae), *Grevillea speciosa* (Proteaceae), *Hakea teretifolia* (Proteaceae), *Hemigenia purpurea* (Lamiaceae), *Leucopogon esquamatus* (Ericaceae), *Persoonia lanceolata* (Proteaceae), *Petrophile pulchella* (Proteaceae), *Phyllota phylicoides* (Fabaceae), *Pimelea linifolia* (Thymelaeaceae), *Pultenaea tuberculata* (Fabaceae). The family Myrtaceae is well represented in the community, but absent from the study, as all locally common Myrtaceae re-sprout following fire and were therefore unsuitable for this study design. All sites were chosen to have minimal *Eucalyptus* cover, such that *B. ericifolia*, *H. teretifolia*, and *Allocasuarina distyla* (not included in our study because it is dioecious) would be the dominant canopy species late in succession, at heights of 3-5 m.

## Field measurements

The study was conducted over a single year, with the initial plant measurements and subsequent harvest conducted during the late autumn and early winter, the period of minimal vegetative growth in this plant community. Repeat visits were made throughout the year to record reproductive activity. Individuals were sampled at different ages across a fire-created chronosequence, from 3 months to 30 years. Site ages were estimated from fire records maintained by NSW National Parks and Wildlife Service. At the conclusion of the study, the approximate ages of the individuals on the six sites were: 1.4, 2.4, 5, 7, 9 and 31 years. Plants were tagged during May-June 2012 and harvested during May-June 2013, with a given individual tagged and harvested within 2 weeks of the same calendar date. Only one species, *P. lanceolata*, displayed any shoot extension during these months. These months are similarly a period of minimal reproductive activity – only *B. ericifolia*, *G. speciosa,* and (occasionally) *H. purpurea* flowered during this period – although a number of species had immature fruit from the previous year (*P. lanceolata*) or small buds that would open in the subsequent year (*B. ledifolia*, *C. ericifolium*, *E. microphylla*, *G. buxifolia*, *L. esquamatus*).

Seven healthy individuals of each species were selected at each site (and thus age). At the beginning of the study year, basal diameter was recorded approximately 10 mm above the base to avoid the basal swelling. At the end of the study year, diameter was remeasured at the same location. Plant height was also measured at the beginning and end of the study year. On each plant, a robust shoot was designated as the leader and the number of leaves along the entire length of the leader was measured. For species with a regular pattern of small leaves, the length of stem covered with leaves was measured in lieu and a species-level “leaves per stem length” conversion factor was applied to convert stem length to a leaf count. At the end of the study, the number of leaves remaining along this same shoot length was reassessed to determine the number of the original leaves remaining. In addition, the length of new shoot extension and number of leaves along the new shoot extension were recorded. At the end of the study plants were then harvested at ground level and oven dried at 60ºC for at least 1 week. Leaves and stems were separated and weighed.

Flowering parts on all individuals were recorded during repeat censuses, every four weeks during cooler months and every three weeks during spring and summer. At each census, all flowering parts were counted, including buds (by size class), flowers, young fruit (by size class), and mature fruit. For some species the size of immature and mature fruit and cones was also measured, as the final size of the structures was quite variable. The exact flowering parts considered varied considerably by species due to their diverse floral structures. Flowcharts detailing what flower parts were included for each species are provided as an Online Appendix accompanying a previous publication (Wenk *et al.* 2017). Total reproductive investment is the sum of investment in all the different flowering parts during the year, tabulated on a dry mass basis (Wenk *et al.* 2017).

## Calculations

Leaf growth is calculated

This represents value is the investment both in leaves to replace leaf loss and leaves to expand leaf area. Leaf turnover, calculated at an individual level, was required to separate the combined leaf growth metric into the two leaf growth components. Shoot leaf loss is calculated as the difference between leader shoot leaf count at the start of the study year and the number of leaves remaining along the same shoot length at the conclusion of the study year. Leaf loss / initial leaf count indicates the proportion of leaves lost along the shoot during the year. It is assumed this ratio applies to the entire leaf canopy to determine the total yearly leaf loss. If total leaf investment is greater than total leaf loss, leaf replacement investment is set as equal to leaf loss and leaf expansion is calculated as:

Leaf expansion investment = Total leaf investment – Leaf replacement investment

If total leaf investment is less than total leaf loss, leaf replacement investment is set as equal to total leaf investment, leaf expansion investment equals zero, and the leaf budget deficit is calculated as:

Leaf budget deficit = Leaf loss – Leaf replacement investment

Stem growth is calculated…

In this manuscript, reproductive allocation is calculated as:

Reproductive investment / Surplus energy

Where,

Surplus energy = Reproductive investment + Leaf expansion investment

1. including stem weight would cause RA to decrease dramatically for most species, because actual stem diameters and weights must continue to increase each year that any shoot growth occurs, even though the volume of functional wood (sapwood) may be stable or declining.
2. *why not stems? Hard. This is first cut. If assume pipep model, will reduce RA, but only for values < 1*
3. We focus on the division of energy investment into leaf replacement, leaf growth, and reproduction, for two reasons. First, leaf mass is a much between predictor of the following year’s total production than is the total standing biomass of the plant. This is because much of the stem biomass is functionally dead heartwood. The mass of heartwood increases ### each year, ###. We are unable to divide the yearly increase in stem biomass into the incremental increase in sapwood versus heartwood. The increase in sapwood mass should reflect the increased
4. *Define surplus energy as leaf expansion + reproduction.*

* Leaf weight was converted to leaf area by applying a species mean leaf mass per area. For each species, leaf mass per area was calculated on leaves collected at three shoot ages from each of ### individuals. Leaves were scanned to determine leaf area and then over dried at 60ºC for 48 hours to determine dry leaf mass.
* Maximum height is the height of the tallest individual included in the study.
* Longivity and age at maturity are also determined based on the study sites.

## Analysis

# Results

###intro sentences###

Although all study species broadly follow the same lifetime developmental pathway of investing first in height and leaf growth and later in reproduction, the magnitude and timing of their investment in different tissues types is diverse.

The apparent shape of RA schedules is enormously affected by which energy pools are tabulated into the *vegetative* versus *reproductive* energy pools (Figure 2). The four panels in Figure 2 plot data for the same species, *Epacris microphylla*, but in each different yearly tissue investment pools are included in the RA calculations. Figure 2a shows an RA schedule when seed weight is used as the proxy for reproductive investment and total vegetative NPP is considered vegetative investment, leading to a low maximum RA. In Figure 2b, all accessory costs are included in the measure of reproductive investment while vegetative investment again considered total vegetative NPP, including both investment to replace shed leaves and stems and investment to increase stem biomass and leaf area. In Figure 2c, the vegetative investment pool is reduced by ignoring stem weight, done because stem investment cannot easily be divided into “replacement” and “expansion” components, since the amount of sapwood being converted to largely inactive heartwood is unknown. Last, Figure 2d depicts an RA schedule based on the narrowest definition of vegetative energy investment, yearly energy invested in expanding leaf area. The sum of reproductive investment and energy investment in leaf expansion are together surplus energy, the definition of tissue investment pools used in theoretical models of RA schedules.

###Transition sentence### For all study species, investment in the seeds themselves was an exceedingly small proportion of total reproductive investment (Figure 3; Wenk &Falster 2017). The remaining reproductive investment is to so-called accessory tissues, including both tissues associated with the flower and fruit of a successfully matured seed and floral and fruit tissue that are aborted without forming a seed. Accessory tissues can be further divided into tissues associated with pollen-attractions (floral investment up to the point of pollination) and tissues associated with the provisioning of a fertilized ovule (packaging and dispersal tissues, including the weight of the seed itself). The relative investment in each tissue category is notable different across species, but a pervasive cross-species pattern is that all species invest hugely in flowers that abort before, at or just after pollination (Figure 3).

Taking the RA formulation shown in Figure 2d, reproductive investment as a proportion of surplus energy, Figure 4 depicts RA schedules for all fourteen study species. Here, species are sorted from top to bottom based on lifespan and age at maturity. Across the species, there were notable differences in all major life history dimensions, with some species disappearing from the community already at quite young ages while others were still thriving at the 32-year old site. Since this community, and other eastern Australian plant communities with a similar floristic composition, are actively managed for a 7-30 year fire interval, few plants have lifespans much beyond 30 years; indeed the oldest site was burnt in a managed fire the year following this study. Species also displayed diverse maturation ages, with three species, *Boronia ledifolia, Hemigenia purpurea,* and *Pimelea linifolia*, flowering with a year of germination and one species, *Persoonia lanceolata* first flowering at age 7.

The shape of the RA schedule, maximum RA achieved, and age at which the maximum RA was achieved were also different across the study species (Figure 4). All species except 1 displayed a continuous increase in RA across their lifespans, with only *Petrophile pulchella* peaking at an intermediate age. Nine of the fourteen species had a maximum RA of 1, indicating all surplus energy is invested in reproductive material. Among these six species achieved an RA of 1 at least two years before dying out, while in the other three RA=1 was only measured among the oldest plants. Across the remaining five species, four reached maximum RA values above 0.75, with only *Petrophile pulchella* having low population level RA values due to many individuals not reproducing in a given year. This variation suggest the species can be categorized as exhibiting different RA schedules, as shown in Figure 1. *Petrophile pulchella*, which reaches a maximum population-level RA at intermediate ages, is designated as having a declining RA schedule (Wenk & Falster 2015). Indeed, the maximum RA reached by these species, designated as the average RA of the plant age class with the highest average RA, is associated with a collection of key life history traits (Figure 5). Across the fourteen study species, maximum plant height, age at maturity, and lifespan are all correlated with each other, such that taller plants live longer and begin reproducing later. Relatively few species do not reach a maximum RA=1, but the species that do are generally taller, longer-lived, and reach reproductive maturity later.

By definition, once RA reaches 1, there is no longer any investment in the expansion of leaf area; the plant has ceased to grow to a bigger size. This means that many of the species had reached their maximum size many years before death and hence were displaying determinate growth. This can be visualized by plotting the actual investment in reproduction and leaf expansion against plant size (Figure 6). All species began with zero reproductive investment and with increased size (and age) reached reproductive maturity and began allocating energy to reproductive tissues. For all species there was an overall increase in reproductive investment with plant size, excepting the individuals of some species that did not reproduce during the study year and plotted in the grey-shaded “no investment” bar at the base of the plots.

All individuals also showed strong investment in leaf expansion in the years preceding reproductive maturity. In some species, including *Grevillea speciosa, Hemigenia purpurea,* and *Pimelea linifolia* leaf expansion fell close to zero – or to zero – almost immediately after reproductive maturity; these are species that rapidly reached RA=1 following reproductive maturity. At the other extreme, some species, including *Epacris microphylla* and *Hakea teretifolia*, showed modest declines in leaf expansion investment following the onset of reproduction, but continued to invest in both across many years. Overall, all species exhibited a cross-over in the magnitude of investment in increased leaf area versus yearly reproductive production across their lifetimes. These different leaf expansion investment trajectories are reflected in these species’ leaf areas trajectories with plant age, with species like *Epacris and Hakea* continuing to increase their total leaf area throughout the chronosequence, while in *Grevillea speciosa, Hemigenia,* and *Pimelea* a maximum leaf area was achieved quite early (Figure 7). Of note, multiple species not only reached their maximum leaf area many years before death, but their leaf area then declined across multiple years although they were still healthy, robustly reproducing individuals (Figure 7).

Ceasing investment in leaf expansion does not indicate these species are not producing new leaves. In Figure 6, the dark green circles indicate yearly investment in leaf replacement – new leaves grown to replace shed leaves. Most individuals invested significant and increasing amounts of energy in leaf replacement with increasing plant size. However, many individuals, especially the larger, older plants did not invest sufficient energy in leaf replacement to fully compensate for leaf loss. In these individuals, the top of the vertical line rising above the leaf replacement investment circle indicates how much energy *should have been* invested in leaf replacement to offset leaf loss. This is most immediately obvious for older *Boronia ledifolia* individuals where many plants had large leaf budget deficits, but older individuals of 10 species displayed a deficit. This leaf budget deficit leads to the declining total leaf area in older plants displayed by *Boronia* and other species (Figure 7). The standing leaf area is of key functional importance to all species, for leaf area, not height, stem diameter, or total plant weight, is the best predictor of total yearly production (Sup Mat Figure 1).

Overall, at the broadest level, all species display a fundamentally identical trajectory of investment in leaves versus reproductive tissues as they grow and age: as seedlings and saplings they invest solely in stem and leaf growth, increasing rapidly in height and leaf area. They have high shoot extension rates and consequently high increase in leaf area per shoot. At some age, notably different across the study species, plants reach reproductive maturity. As the plants initiate investment in reproductive material and subsequently increase allocation to reproduction, they all show a marked decline in relative investment in leaf growth. Notably, for ten of the 14 species, total leaf area declines year-upon-year starting at some age. That is, these species have negative leaf growth for part of their lifetime, as they fail to replace all leaves shed during the year.

1. in our species height growth does not stop with age – just tapers
2. initial leaf weight is a good predictor of NPP

# Discussion

* The vast majority of perennial plants are iteroparous, having multiple reproductive episodes during their lifetime.
* For almost all species height continues to increases throughout life, even though for most species leaf area asymptotes within a few years of reproductive maturity and often declines for several years before an individual’s death.
  + cannibalizing existing leaf area to increase investment in reproduction (to me, an evolutionary, not ecological argument; it is the ratio of leaves:flowers that leads to this)
* In many annual species, a plant reaches its maximum size before the onset of reproduction, then allocating its energy to seed production. The plant may survive for many additional months, as it reproduces. This is not so different from some of the shorter-lived perennial species, where they achieve their maximum leaf area (or nearly reach it), by the time of reproductive maturity. They then enter a prolonged period of reproductive investment, building at most enough leaves to replace shed tissues. For some species, it appears that their continued leaf production is simply the default construction of leaves at flowering nodes, as they have turned their attention solely to reproduction. Individual plants may persist for multiple years in this life stage – and indeed from an evolutionary perspective, a prolonged period of slow decline may be how they achieve sufficient seed output – but they are most certainly not displaying indeterminate growth.

# Conclusions

* Unclear if the allocation processes leading to declining leaf area are intentional or the result of allometric constraints.
  + In many species, number of flowers formed is most strongly correlated with total leaf production (replacement + expansion), not surprising since flowers form in the axils of newly formed leaves or as a cluster at the top of near growing shoot. (plot of “ovule\_count” vs “all\_leaf\_inv”)
  + For these species, there is leaf expansion, if, after replacing shed leaves and producing the accompanying number of flowers (and fruit), there is energy remaining for addition leaf growth (and yet more reproduction)
  + Alternatively there is leaf contraction, if there is not sufficient energy to replace all leaves, given the simultaneous expenditure on reproduction.
  + This would be a sensible evolved strategy, with plants rapidly accumulating significant leaf area prior to reproductive maturity, but with the onset of reproduction, species have evolved to produce a number of flowers (per leaf produced) that uses the majority of energy produced, such that there is little leaf expansion. Functional traits, especially leaf turnover rates, fine tune this process, such that different species cease leaf expansion at different times relative to reproductive onset. Traits associated with aging plants that cause productivity to decline, slowly shift the balance in the direction of leaf area loss.
* What about species that do not seem to have reproductive output driven by yearly leaf production?
  + Also, reproductive investment most closely correlated with total plant weight (quite strong correlations), even though total energy production most closely correlated with leaf area

- why plants cannibalising leaf. Actually not that unlike herbs, just over longer time period.

- how wide spread is this?

- growth often considered via stem diameter, likely to miss asymptotic production of leaf

* Declining leaf area with age/size
  + notable how many species show a decline in leaf area within a year of initiating reproduction.
    - These species display a bang-bang schedule, but continue to survive for a number of year’s post reproductive onset, just with a continued year-upon-year decline in leaf area.
  + Two of the four species that are dominant canopy members late in succession, *H. teretifolia* and *P. pulchella*,are the only study species that continued to demonstrate strong investment in leaf area at 30 years of age. The other two canopy species, *B. ericifolia* and *P.a lanceolata*, have negative investment in leaf area at the oldest site, with many *P. lanceolata* individuals displaying minimal growth.
  + There are two additional understory species that continued to be common within the oldest site, *Leucopogon* and *Epacris*, both members of the heath family (Ericaceae). Although leaf investment for both these species was much lower at the oldest site, many individuals of both species did continue to exhibit positive leaf investment together with high reproductive investment.
* Leaf area is a better predictor of future investment than is total plant weight. Since leaf area represents the plants photosynthetic capacity, this should be no surprise.
  + For many species both are fairly poor, but more or less across the board, leaf area is better
  + This means that is total leaf area is declining with size, plants are on a slow trajectory to death as soon as they begin cannibalizing leaf area to support reproductive investment
* It is widely assumed that large perennial species continue increasing in size throughout their lifetimes. If leaf area is used as the measure of plant size, this is not true for these study species.

- Many authors acknowledge that reproductive investment is an understudied sink contributing to declines in various plant growth measures (i.e. GPP, NPP, leaf area) with age (Ryan *et al.* 1997; Day *et al.* 2001; Tang *et al.* 2014). A growing collection of literature has stand-level carbon-flux data, demonstrating that older stands have declining GPP. Some of the growth-related explanations for this decline, such as hydraulic limitation with increasing height and declining photosynthetic rates with plant age (refs###) are unlikely to be influenced by reproductive investment. Others, including declining leaf area and declining height growth (increasing competition) with age, may be a direct response to increased reproductive allocation (Ryan *et al.* 1997; Becker *et al.* 2000; Genet *et al.* 2010). Only with detailed individual or stand-level data on

It is generally assumed that plants first pay the cost of replacement tissues and then divide the remaining, surplus, energy into components including leaf growth and reproduction. For the current collection of plants this was not observed for many individuals, where a plant continued to reproduce robustly, but failed to invest sufficient energy in leaf growth to fully offset shed leaves (Figure 6). This year-upon-year cannibalism of leaf area, ostensibly to support reproductive output, suggests some energy allocation decisions are independent of actual energy supply. This could be true, for instance, of species where buds form at all leaf nodes on new shoots. The leaf growth would be replacing shed tissue, but so many buds may be initiated in the process that a plant would

Many of the study species show a linear relationship between total leaf investment (replacement and expansion) and reproductive investment once most plants in the population have reached reproductive maturity (Supp Mat). These are primarily species where flowers form in each leaf axil or species where an inflorescence forms at the tip of each growing shoot, both growth patterns where it is expected for total leaf growth and reproductive investment to occur as a pair.

For A. saccharum, age (which varied from 30 to 160 years) was a significantly better predictor of LAI decline than dbh. (Nock *et al.* 2008)

Calculating RA as a proportion of surplus energy makes the implicit assumption that a plant is first investing a proportion of net primary productivity (NPP) to maintain its current size and then divvying the remaining energy into fractions allocated to reproduction versus increased vegetative size.

(Ryan & Waring 1992) - Stem maintenance and stand development in a subalpine lodgepole pine forest Ecology

Sala: “Another area of study that has focused on C as a primary driver of productivity is the research that has been done on the mechanisms underlying the well-known age- and size-related growth declines in trees and forests (Ryan *et al.* 1997). The two classical groups of hypotheses to explain size/age-related declines of growth rates in trees and stands focused on the extent to which growth declines occurred as a result of reduced C supply via photosynthesis (assimilation hypotheses) or an increase of C demand due to increases of the respiratory load (respiration hypotheses; see (Sala, Fouts & Hoch 2011 p. 2) for a discussion). However, both of these groups of hypotheses rest on the same assumption: that growth declines are due to limiting C availability. This perspective is now questioned based on current research indicating that alternative factors (e.g., turgor, long-distance transport of assimilates, nutrients) contribute to growth limitations in tall trees (Woodruff, Bond & Meinzer 2004; Woodruff, Meinzer & Lachenbruch 2008; Sala & Hoch 2009).”

the decrease of light capture efficiency in mature trees (Niinemets, Sparrow & Cescatti 2005) or the decrease of the turgor pressure limiting cell expansion and reducing the carbon sink of growth (Woodruff et al. 2004). – from (Genet *et al.* 2010)

Becker et al. 2000 –shouldn’t assume hydraulic limitations main reason height growth ceases

In adult trees, allocation of photosynthate to flower and seed production has priority over primary and secondary stem growth (Oliver & Larson 1996). (Becker *et al.* 2000)

On very poor sites, heavy seed production can even eliminate annual ring production (Matthews 1963). – in Becker 2000

“When height growth ceases to offer a competitive

advantage through avoidance of shading, then

(genetically programmed) resource allocation will be

adjusted to enhance tree survival and reproduction,

not necessarily wood production.” Becker 2000

large fruit crops may decrease vegetative growth during both fruiting and non-fruiting years because of energy reserve depletion (Dickson 1991) – in Becker 2000

Declines in leaf area in older trees have been noted by different measurements including increased canopy gap fraction (Quinn & Thomas 2015), decreaing LAI (Nock *et al.* 2008), ###. In addition, older trees have a lower ratio of branch biomass to main stem biomass than do younger trees (Liu, Fox & Xu 2002; Lehtonen *et al.* 2004)

(Sheil *et al.* 2017): So why would growth ultimately decline? …and

The proportion of intercepted energy invested in stem growth declines with size (Kaufmann & Ryan 1986; Thomas 2010).

Plants in low light conditions has lower RA - (Delerue *et al.* 2013)

Reproductive investment can also be summarized by reproductive value (RV) curves that plot total yearly reproductive investment against plant size and show, for most perennial species, that reproductive investment increases with plant size, asymptoting as plant’s age (Weiner *et al.* 2009). RV curves do not however depict the growth-reproduction trade-off, for they consider a plant’s size not vegetative growth. In these species, it is total

Overall, if the proportion of NPP going to maintenance costs increases sharply with age or NPP does not keep pace with increases in leaf area and stem architecture, the surplus energy pool may rapidly asymptote – or even decline – impacting growth, reproductive investment, and potentially RA. RV curves suggest that although reproductive investment plateaus with plant size, it rarely declines. In addition to the outcome of the growth-reproduction trade-off this reflects simple allometric constraints for many species: the number of buds initiated is often closely linked with the deployment of leaves. Since flowers to the point of pollination can account for the majority of reproductive investment (refs###), many species reproductive investment will be closely linked to investment in vegetative growth, both maintenance costs and new growth. The increasing maintenance costs and reproductive investment, suggest that the pool of energy available for vegetative growth to increase plant size may be severely curtailed long before a plant approaches its end-of-life.

These factors, and others, have been addressed in theoretical models, seeking to understand how the optimal allocation of energy to growth versus reproduction shifts as an individual grows and ages and under what conditions the outcome is indeterminate growth and multiple reproductive events (###).

However, with increasing plant size, the energy expenditure to replace shed leaves and stems, becomes an ever larger proportion of a plant’s total energy budget. While the proportion of *surplus energy* allocated to reproduction may continue to increase throughout a plant’s life, the proportion of its pre-maintenance energy pool may display a different trajectory. A more inclusive calculation of RA presents several potential benefits. First, it presents a more holistic view of energy expenditure to vegetative versus reproductive materials, presenting the possibility that reproductive investment increases as a proportion of total energy investment rather than as a proportion of surplus energy. Phrased alternatively, a plant may be making decisions of energy allocation to reproduction versus vegetative material based on its energy pool prior to replacing shed tissue. This in turn allows for the possible outcome that a plant’s vegetative mass ceases to increase at some size or age, as tissue replacement becomes so high that it consumes the entire energy budget for vegetative tissues. In such instances, the plant should be categorized as displaying determinate growth, yet, contrary to convention, would not have reached its end of life. In more extreme instances, a plant’s reproductive investment may be sufficiently large to leave insufficient energy to replace all shed tissues, leading to a *decrease* in plant size year-upon-year. Decreasing leaf area cannot be a long-term strategy, but may allow a plant to have higher reproductive output for several years at the end of its life. If a plant ceases to increase its vegetative size, RA, calculated as a proportion of *surplus energy* reaches 1 and if a plant’s vegetative size declines, RA exceeds 1. If plants display such growth trajectories, the conventional RA calculation will not accurately depict a plant’s energy allocation decisions.

A few species display declining RA schedules, with a decrease in RA among the oldest plants, interpreted as a mechanism for older plants to survive until their habitat becomes more favourable for high reproductive production and growth (Kohyama 1982; Nakashizuka, Takahashi & Kawaguchi 1997; Ehlers & Olesen 2004).

* PROBABLY NEED A PARAGRAPH LISTING SOME INFORMATION ON WHAT DIFFERENCES WE EXPECT ACROSS SPECIES. RIGHT NOW JUST INCLUDED IN HYPOTHESES
* (Thompson & Stewart 1981) – call RA measure of greatest importance as well
* (Primack 1987)

# Acknowledgements

# Authors’ contributions

# Data accessibility

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# Figures and tables

Table 1: Details on study species and their life history traits

Figure 1: Some possible reproductive allocation schedules. After Wenk & Falster 2015.

Figure 2: Including different biomass components has strong effects on estimates of reproductive allocation. Shown are same estimates for single species *Epacris microphylla*. See sup matt for further examples.

*Inclusion of different energy pools results in very different RA schedule shapes and magnitudes. All 4 panels plot data from the same species, but considering different energy pools: a) All vegetative materials, both new tissue investment and investment to replace shed tissues, are included in the vegetative components, while seed weight is used as a proxy for reproductive investment; b) All vegetative materials are included in the vegetative components and all tissues associated with reproduction (seed + accessory costs) are included in the reproductive investment pool; c) Investment in leaves to increase leaf mass and replacement of shed tissues is included in the vegetative component and all tissues associated with reproduction (seed + accessory costs) are included in the reproductive investment pool; d) Just investment in leaves to increase leaf mass is included in the vegetative component and all tissues associated with reproduction (seed + accessory costs) are included in the reproductive investment pool.*

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Figure 3: Reproductive allocation schedules for the fourteen study species.

*Panels RA schedules, calculated as a proportion of surplus energy, for the fourteen study species. The burnt orange color indicates the proportion of surplus energy to reproduction, while the pale green is the proportion of surplus energy invested in leaf area expansion. The black circles indicate data points for the individual plants assessed. Top to bottom species are sorted from shorter to longer-lived. Rows 3 versus 4 are distinguished by species that approach their maximum RA long before the end of the study sequence versus only near the end of the study sequence.*

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Figure 4:

Plant investment in reproduction (red), replacement of shed leaves (dark green), and growth of additional leaves (pale green). A sizable proportion of net production is investment in the replacement of shed leaves throughout a species’ lifespan, but in many species insufficient energy is invested to compensate for all shed tissue. For individuals where the weight of shed leaves is greater than the investment in replacement leaves, the straight line indicates the magnitude of the deficit; to fully replenish shed leaves the plant would have to exhibit the investment indicated by the top of the line, but instead invests the amount represented by the dark green dot. Investment in reproduction continues to increase with increasing plant weight. Investment in additional leaves declines quickly after reproductive maturity is reached, dropping to zero in many species.

Changes

Daniel fix x-axis formatting (scientific notation

Figure 5. Standing leaf biomass plateaus and even declines with increasing plant age. This trend is stronger in shorter lived species. (Color indicates age, shading from pale blue in the youngest plants, through darker blues to grey and black.)

* try without colours? No longer necessary if age on x axis – *tried and didn’t immediately like it – somehow harder to see progression*

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# Supporting information

Figure: Distribution of biomass allocated to reproduction among different accessory tissues. ?

Like Figure 2 but for all species.