CHAPTER 5.

REPRODUCTIVE OUTPUT: THE INFLUENCE OF LOCAL DENSITY AND FLORAL VISITATION ON FRUIT AND SEED PRODUCTION

5.1 INTRODUCTION

Many sexually reproducing plants, particularly those that are strongly self-incompatible, rely on pollen vectors for mating opportunities. Self-compatible species may escape complete reliance on external vectors for reproduction by employing automatic self-pollination, but for self-incompatible species fruit and seed set is particularly susceptible to the vagaries of pollinator behaviour. Pollination is the precursor to fruit and seed production but successful fertilisation and consequent fecundity is moderated by a number of factors. These include influences on pollen delivery (quantity and quality) such as visitation rate and within-patch foraging behaviour, maternal investment in fertilised ovules as a function of female choice (Stephenson & Bertin 1983), resource allocation and resource availability (Lee 1988) as well as constraints imposed on realised fecundity by herbivory (Hendrix 1988; Mothershead & Marquis 2000) and seed and fruit predation (Lee 1988). Local floral density can regulate the capacity of these components to contribute to fecundity and there is much ecological interest regarding density effects on these processes and ensuant fruit and seed production (Roll *et al.* 1997; Knight 2003). Negative effects of low density on reproduction (commonly termed the "Allee effect", see Groom 1998; Hackney & McGraw 2001) have often been observed for zoological systems, and are likely to be equally important for plant reproduction (Knight 2003).

The response of pollinators to local conspecific density can be important in moulding fecundity because visitation rates and pollinator behaviour can influence both the quantity and quality of pollen delivered to flowers. According to optimal foraging theory, visitors will tend to preferentially visit patches with high floral density because within-patch flight distances are short and resources can be procured at low energetic cost to the forager (see Molina-Montenegro & Cavieres 2006). Sparse patches that proffer few flowers are likely to be less attractive and/or offer fewer floral rewards (Kirchner *et al.* 2005) and indeed, visitation rates tend to be higher to dense populations or patches relative to those that are sparse (Lamont *et*

al. 1993; Forsyth 2003). The extent to which fruit and seed set is influenced by these dynamics, can be contingent on a plant's breeding system. Kunin (1997b) compared the self-incompatible *Brassica kaber* and the self-compatible *Senecio jacobaea*; for both species marked density effects occurred in terms of both visitor quantity (higher visitation rates to dense arrays) and quality (reduced flower constancy (see below) in sparse arrays). The result for the self-incompatible *B. kaber* was a decline in fecundity for sparse plants but no visitation-related effects on fecundity were observed for the self-compatible *S. jacobaea.* Kunin's (1997b) study exemplifies how breeding system coupled with visitation can drive reproductive output, and illustrates the reproductive hazards that self-incompatible species may suffer under density-dependent pollinator limitation. In this case the relationship between visitation and fecundity was readily characterised, but results of investigations into these relationships are often unclear. The complex nature of insect-mediated pollination (which often extends beyond the simple relationship of "more visitors equals more pollen") coupled with the effects of extraneous influences (e.g. resource limitation) can hamper interpretation of visitation-related fecundity responses to density; moreover, many of these components can be spatially and temporally variable.

In addition to the influence of patch density on visitation rate, the dynamics of pollen deposition *within* patches can be influenced by a suite of factors which can affect foraging behaviour (see Kunin & Iwasa 1996) for example; within-patch plant spatial distribution (Goverde *et al.* 2002; Ishihama *et al.* 2006), facilitation/competition effects among conspecifics (Steven *et al.* 2003), individual plant and inflorescence size (Schmitt 1983) and heterospecific species flowering within the patch. In the presence of heterospecific flowers, floral constancy in sparse arrangements can be compromised as visitors can be less faithful to the focal species (Ghazoul 2005b). This may not only reduce visitation to the focal species, but may also reduce conspecific pollen loads carried by visitors (the dynamics of pollen carry over) (Handel 1983) and increase the incidence of stigma clogging with foreign pollen (Rathcke 1983). Alternatively, the presence of flowering heterospecifics can be facilitative. Gross *et al.* (2000) for example, found that fruit set in *Dillwynia hispidia* was positively influenced by an overlap in flowering with *D. uncinata* and *Pultenaea densifolia.* Furthermore, as found in the present study (Chapter 4) and by Bosch & Waser (1999) the number of flowers visited on an individual can vary with density; visitors tend to visit more

flowers per plant on relatively sparse or isolated individuals. Clearly, this can have negative consequences for a self-compatible plant as it can promote disadvantageous geitonogamous pollinations, which Ghazoul *et al.* (1998) observed for the forest tree *Shorea siamensis* (Dipterocarpaceae). All of these factors serve to shape the dynamics of pollen delivery and can differentially influence reproductive effort. Moreover, not all visitors provide an equivalent pollination service and visitor composition can be an important consideration.

Different types of visitors can vary in their effectiveness as pollinators (Brunet & Sweet 2006). For instance, Kirchner *et al.* (2005) found a wide range of visitors to *Centaurea corymbosa* (Asteraceae), but only the bee and butterfly component affected fertilisation rate. Furthermore, temporal variation in flower production both within and between seasons can influence visitor abundance, visitation rates and guild composition, which can variably influence reproduction. Total visitor abundance is an important factor. If a population is saturated with visitors, then it is unlikely that density effects on visitation and subsequent reproduction will be revealed because pollination may not be limiting. Schmitt (1983) found this to be the case for *Senecio integerrimus*. Conversely, the same may be true if pollinators are very scarce in a system (see Bruna *et al.* 2004). Furthermore, pollinator limitation can be scale dependent as exemplified by *Coffea arabica* (coffee) in an agroforestry system (Veddeler *et al.* 2006).

Further complicating the situation is the fact that influences beyond the pollination process, such as resource limitation, can also mould fecundity. Maternal investment of resources to fruit and seed production can be dependent on resource availability as found by Lee (1989) for *Cassis nictitans*. Bruna *et al.* (2004) suggest that low visitation rates to the hummingbird pollinated *Heliconia acuminata* were almost certainly contributing to the very low fruit and seed set observed for this species, but that limited resources could also have been a contributing factor. Similarly, Allison (1990) proposes that variation in seed set in the wind-pollinated *Taxus canadensis* resulted from a combination of both pollen and resource availability. According to Vaughton (1991) pollen and resource limitation are unlikely to be mutually exclusive since their relative importance is likely to be both spatially and temporally variable. However, the theory is stalled by a lack of studies that investigate both factors concurrently, adding to the uncertainty surrounding their relative contribution (Ghazoul 2005a). Furthermore, herbivory and fruit and seed predation can negatively influence

reproduction and can also be density dependent. Reduced fecundity in *Collinsia torreyi* was attributed to herbivory, perhaps in concert with low resource availability (Parmesan 2000). In this instance, herbivory was density-dependent, however, generalisations regarding herbivory responses to host plant density are difficult to make; Kunin (1997b) describes the response pattern as "noisy and inconsistent". Isolated plants of the self-incompatible *Cistus ladanifer* suffered reduced pollination success which was further compounded by higher levels of fruit predation (Metcalfe & Kunin 2005). These studies illustrate the importance of considering multiple factors when investigating density effects on net reproduction.

As outlined above, seed and fruit production can be variously dependent on a number of factors. Nevertheless, it remains that visitation rate and behaviour is often influenced by local density as was apparent for *W. luteola* and *D. sieberi* in the present study (see Chapter 4); moreover, density-dependent visitation has been subsequently correlated with reproduction (e.g. Silander 1978; Steven *et al.* 2003; Molina-Montenegro & Cavieres 2006) (but see Bosch & Waser 1999; Bosch & Waser 2001). Despite the difficulty in establishing the specific effects of all possible components, quantifying female fecundity remains a relatively straightforward variable to assess. Quantitatively assessing fecundity is commonly undertaken by measuring fruit and seed set against flower production (see Aizen 1997; Bruna *et al.* 2004). The result is a set of ratios i.e. fruit to flower (FR: FL), seed to fruit (S: FR) and seed to flower (S: FL). Factors such as the level of fruit and seed abortion and predation can (and should, depending on the questions being asked) be integrated into these ratios as appropriate. Results can be consistent across all three ratios; for instance Caruso (2002) found consistently non-significant density related results for S: FR and FR: FL in *Ipomopsis aggregata*. However, inconsistent results across the ratios have also been found (see Aguilar & Galetto 2004). It is therefore prudent to measure all three, as they represent several steps along the reproductive continuum (Dudash & Fenster 1997).

5.2 Aims

In this chapter, reproductive effort of the three study species is quantified with the aim of determining whether female fecundity is influenced by local density and to what extent it may be shaped by visitation rate to flowers.

5.3 METHODS

5.3.1 *T. australe* FR: FL and S: FR ratios

In the 2004/2005 *T. australe* flowering season, up to five stems per plot were tagged at ABR and MOR to determine FR: FL ratios from open visitation. Focal plants were the unit used for determination of ratios. Mostly, five stems were chosen on the plant, but in the cases where these were not available, the number of stems was augmented using a near neighbour. Stems were chosen based on a high level of health, in the hope that they would maintain vigour over 6-8 weeks of repeated data collection for replication purposes. Stems were chosen based on whether they possessed vigorous "growth tips" which were producing buds and flowers. It was found that growth was checked by a lack of water and the growing tips were the first part of the plant to suffer dieback under adverse conditions. Because of the drought conditions the Northern Tablelands experienced during the study period, fewer replicates were taken than intended before plants succumbed to water stress, or at least lost their growing tips and therefore the marked stems. Data collection was repeated at MOR in November 2005 when flowering recommenced, but was again hampered by dry conditions.

Fruits can be somewhat persistent along the lengths of the stems of *Thesium*, however, more often than not, the fruits drop and a scar remains. Although this is a good indicator of fruit production, there are many occasions where a fruit aborts before maturation. It was not known if aborted fruits leave a similar scar to those left by fully mature fruits, hence, a fruit to scar ratio calculated on that basis may have overestimated fecundity. In light of this, the method used took into account the progression of all reproductive structures along the stem. Stems were marked with red cotton thread tied at the base of the axil of the last (and oldest) persistent structure encountered along the stem and above the first scar. Therefore, the section of stem under investigation had a reproductive structure persisting at every axil (immature and mature fruits, non-developing fruits, flowers and buds (Figure 5.1)). Stems were then mapped for reproductive structures. Monitoring took place at intervals of 1-2 weeks to determine the fate of the reproductive structures.

Fruit collected from sparse and dense plots at the ABR and MOR sites in March 2005 were used to investigate *T. australe*'s S: FR ratio, including fruit resulting from stems bagged for autogamy assessment and open fruit randomly collected within the plots. Fruits were taken to the laboratory where they were opened for the purpose of scoring seed set. *Thesium*

australe produces a single-seeded drupe and on opening the fruit, it was found that the level of seed development was variable. Seed fill within fruits ranged from 100% to a complete lack of seed and partial fill varied between these extremes (Figure 5.2). Therefore, S: FR ratio measurements took into consideration the percentage of the fruit filled with seed, which was assessed to the nearest 0, 25, 50, 75 or 100% seed occupation of fruit. Seed to flower ratios (S: FL) were not quantifiable for *T. australe* due to the difficulties in collecting material, which meant that FR: FL and S: FR ratios data were obtained from different material and seed counts could not be related back to the flowers from which they arose.

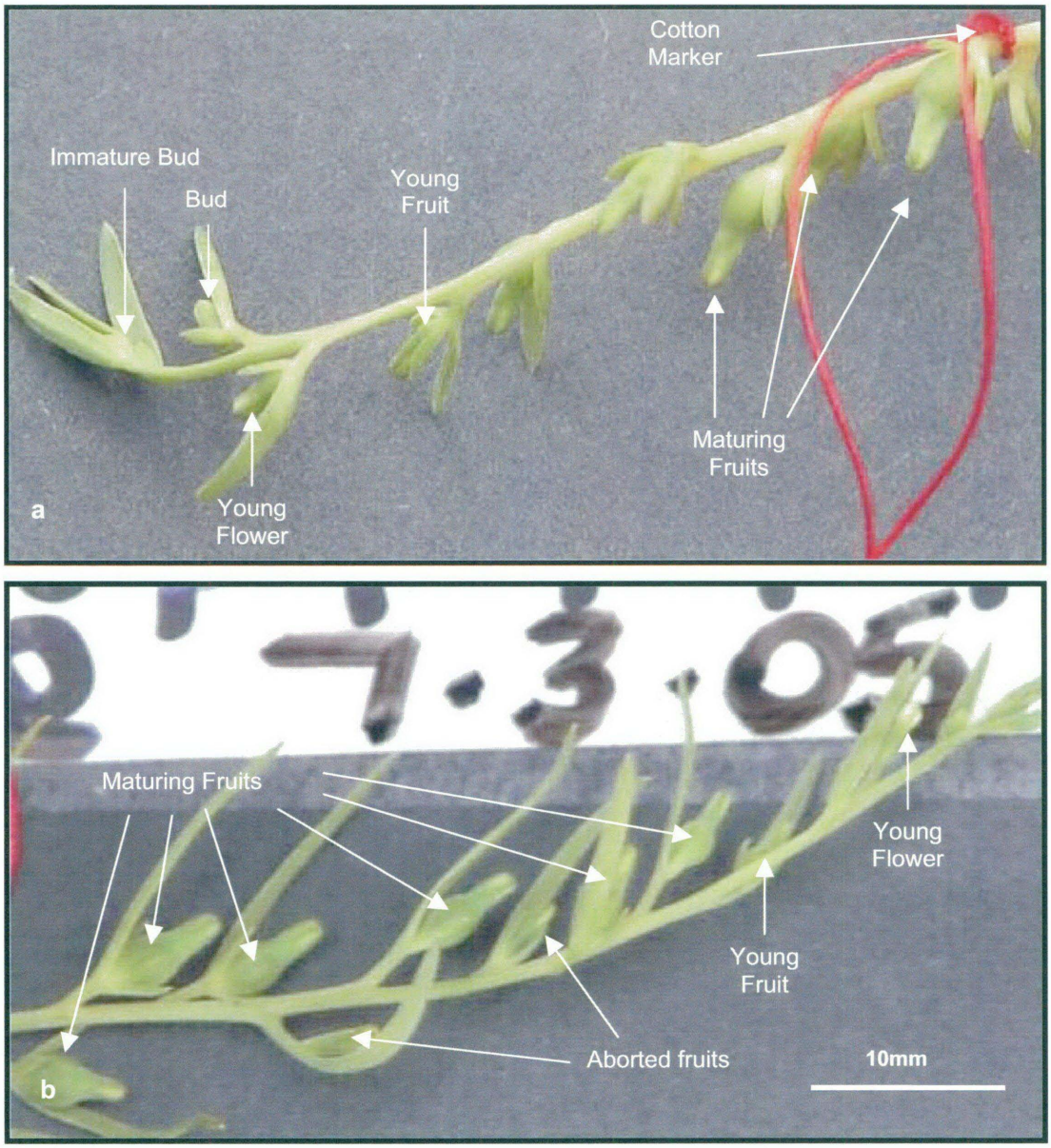

Figure 5.1 *Thesium australe* stems tagged for FR: FL ratio monitoring **a)** indicating reproductive structures **b)** and indicating aborted fruits.

5.3.2 *W. luteola* FR: FL, S: FR, S: FL and Seed Abortion

In 2004, to assess FR: FL and S: FR ratios, flowers on *W. luteola* focal plants in sparse and dense plots were tagged as they arose on the plant and enclosed in bags (see Chapter 3 for bagging methods). Bags were collected when fruit had matured and they were then placed in paper bags and stored on silica gel until processing could be undertaken in the laboratory. Often fruits arrested during development, and were void of seed or contained seed that had aborted (appearing flat, dull and withered). A fruit was therefore defined as containing at least one "healthy " seed. Bags were emptied into a grid-lined petri dish to facilitate counting under the stereomicroscope and the number of healthy and the number of aborted seeds were counted for each fruit. After removing fruit debris, the sample was weighed. The weight of an individual seed contained in a given fruit was estimated by weighing the entire sample of "healthy" seeds (i.e. aborted seeds were removed), and dividing this weight by the number of seeds counted.

Individual plants produce few flowers, and further data collection was undertaken in 2005 at the plot level to increase sample sizes. To assess FR: FL and S: FR ratios, up to twenty flowers were randomly tagged within sparse and dense plots at three sites. Flowers were bagged, (see Chapter 3) when petals had wilted. To supplement this material, a further 5-15 fruits were randomly bagged within plots, to counter possible loss of bags and ensure adequate replication. Bags were collected when fruit had matured and stored on silica gel until processing could be undertaken. All bags collected were assessed for fruit set.

Furthermore, a subsample of 10 bags per plot was used to assess seed weight as a surrogate for S: FR ratio. This was employed as a more time-efficient indicator of seed set than counting individual seeds. Any bags that were damaged were not included. Seed: fruit ratios were also extracted from these data; this involved estimating the number of seeds contained in a given fruit. To do this, the total seed sample contained in a given fruit was weighed and then divided by the average weight of an individual *W. luteola* seed (which had bee previously calculated); on average, an individual seed weighs 2.5×10^{-5}g (see Chapter 6 - Results Section 6.4.1.2). (Thus the sampling unit was the fruit, rather than the individual seed). This also allowed for S: FL ratios to be estimated for the 2005 material.

5.3.3 *D. sieberi* FR: FL, S: FR, S: FL, Seed Abortion and Predation

Reproductive output was assessed for *D. sieberi* in 2004 and 2005. In 2004, to assess whether natural FR: FL ratios differed between densities and sites, 5 stems on each focal plant (5 x sparse plots, 5x dense plots, 3 x sites = 30 focal plants) were tagged using coloured electrical wire. On each stem, the number of buds, flowers and developing fruit were scored. The stems were re-visited regularly to monitor development until the stems had finished flowering. Stems were bagged and collected when fruit had matured (January 2005) so that fruit and seed set could be scored in the laboratory. This method allowed for flowers to remain available to pollinators throughout the flowering period however, it proved time-consuming and when ratios were reassessed in 2005, a simplified method was implemented.

In 2005, the method provided a "snap-shot" assessment of fruit set, rather than tracking the fate of individual flowers over the flowering period. At least 3 stems in each plot at the three study sites were bagged in October 2005 when flowering was at its peak (Table 5.1). Before bagging, unopened buds and developing fruit were removed from the stem, leaving only fresh and withering open flowers. The number of flowers on the stems was counted and a small piece of coloured rope was inserted for identification before the bag was secured. Bags were collected in January 2006 after fruit had matured (but when most were predehiscent) and were taken to the laboratory for scoring. Fruits were scored for seed abortion (fruit that contained only small, wrinkled seed), number of seeds per fruit (undehisced fruit) and predation (Figures 5.3 & 5.4). Seed to flower (S: FL) ratios were also calculated using the 2004 and 2005 material.

Many *D. sieberi* fruits were found to house Coleoptera larvae that were consuming seed. In addition to scoring for predation in fruits on stems bagged for FR: FL in 2004/2005, three stems per focal plant at each of the sites were bagged to specifically assess predation; these were collected when fruits were mature but predehiscent in January 2005. Bagged stems were collected in paper bags and stored with camphor until scored (in an attempt to arrest seed consumption by the larvae). The camphor had little effect however and larvae survived within undehisced fruits. When fruits were counted, it was found that some had dehisced within the bags; these were separated from the fruits that were opened manually. Predation was scored based on the presence of live insect larvae or if holes were apparent in the integument of dehisced fruits (Figure 5.3).

Table 5.1 Number of plants, stems and flowers assessed for *D. sieberi* FR: FL ratios in 2005. For all sites there were 5 dense and 5 sparse plots assessed with the exception of OAR where four dense and five sparse plots were assessed.

Site	Plot Type	# Plants	Total # Stems	Range of Flowers/ Stem	Total # Flowers	Mean Flowers/ Stem ±SE
POW	Sparse	20	21	2-25	286	13.6 ± 1.5
	Dense	20	20	8-40	383	19.2 ± 2.2
MOR	Sparse	18	19	7-60	444	23.4 ± 3.1
	Dense	20	20	12-55	488	24.4 ± 2.9
OAR	Sparse	12	20	14-54	470	23.5 ± 2.2
	Dense	16	16	13-50	439	27.4 ± 3.2

5.4 Statistical Analyses

Analyses were undertaken using Statgraphics® *Plus* Version 5.1, 2001. One-way ANOVAs were employed after data were checked for normality using Cochran's test. In addition, standard kurtosis and skewness values were checked to confirm normality. Non-normal data was transformed as necessary before analysis. Tukey's (HSD) was used post-hoc to discriminate among the means. If data could not be adequately transformed and normalised, a Kruskal-Wallis non-parametric comparison of the medians was undertaken; differences among medians were determined using box and whisker plots (median notch) as generated by the software package. Fruit predation data was assessed using multifactor ANOVA (Type III sums of squares was employed, allowing for an unbalanced design and for the effect of each factor to be assessed independently of other factors included in the model). Chi-square analysis was used to investigate the frequency of percent seed-fill, which varied within *T. australe* fruits. Simple linear regressions were used to investigate relationships between fruit and seed production and visitation rates.

Figure 5.2 *Thesium australe* fruits, which were void of seed; red circle indicates a fruit containing partial seed-fill.

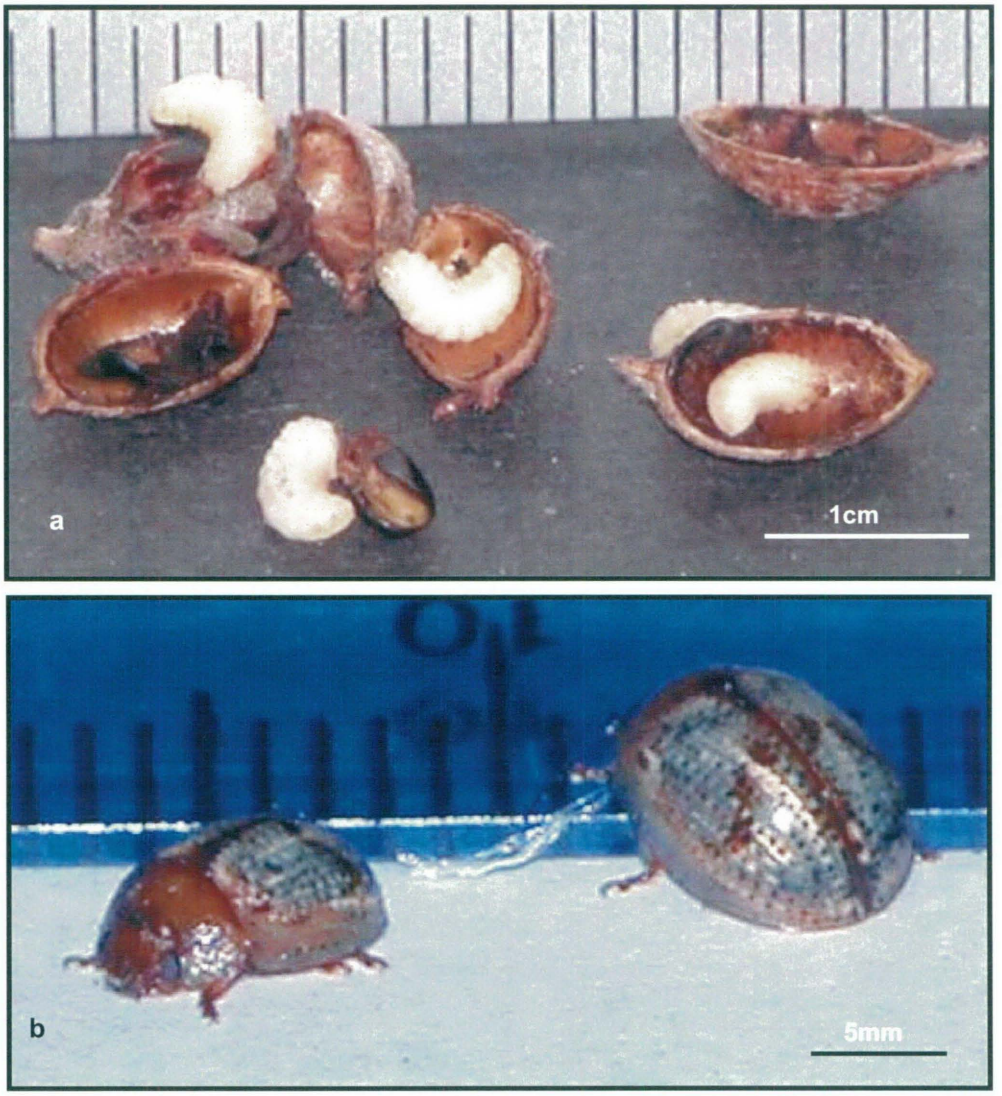

Figure 5.3 **a)** Insect larvae found within *D. sieberi* fruits **b)** Coleopterans which had developed in *D. sieberi* exclusion bags; the likely source of the larvae.

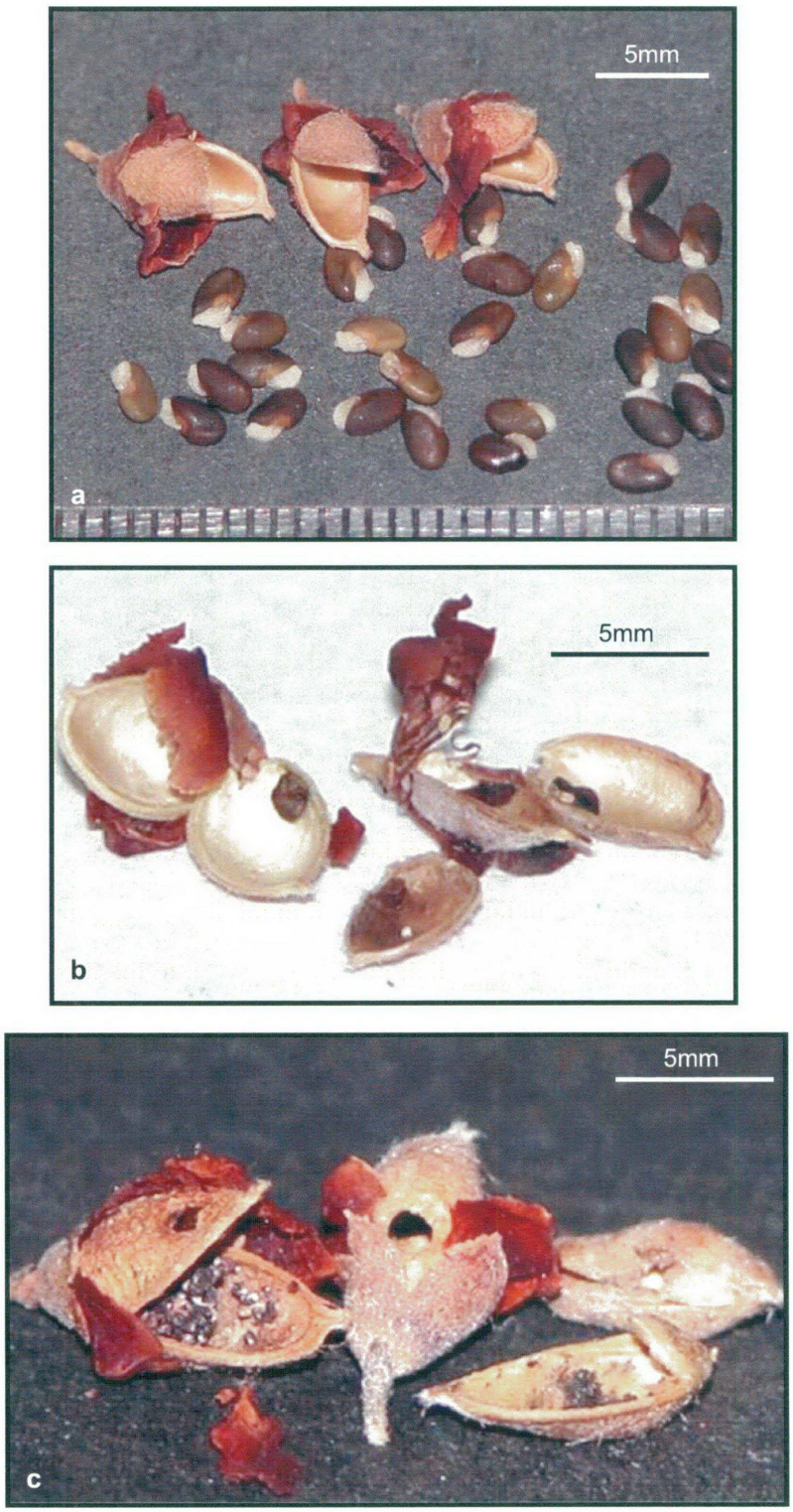

Figure 5.4 *Dillwynia sieberi* fruit and seed **a)** healthy fruit and seed **b)** fruit containing aborted seed and **c)** fruit showing obvious signs of predation.

5.5 RESULTS

5.5.1 Reproductive Output

5.5.1.1 Thesium australe

FR: FL Ratios

Fruit to flower ratios for *T. australe* under open natural conditions did not vary between densities within either site in 2004 (ABR $F_{1, 47}$ =3.56, P= 0.065; H=2.767, P=0.096 comparison of means) or for MOR in 2005 ($F_{1,48}$ =0.00, P= 0.993). When densities were pooled for site, FR: FL was significantly lower at ABR compared with both MOR years (H=18.01, P=0.000 comparison of medians) (Figure 5.5). This would indicate lower reproductive output at ABR however, the S: FR ratio (below) is a more comprehensive assessment of this. The open FR: FL for ABR (0.65±0.047) is similar to that calculated for autogamous fruit set at this site (0.69±0.09, see Chapter 3), which indicates the possibility that the majority of fruit produced at this site overall, was produced autogamously. (Data for MOR was insufficient for analysis, but an autogamous FR: FL ratio of 0.63 was observed based on only four stems and six axils).

S: FR Ratios

When densities were compared, S: FR ratio, based on all fruits that contained at least 25% seed fill or more (i.e. all % seed fill) was significant only at ABR ($F_{1, 446}$=6.57, P=0.012; MOR $F_{1,199}$ =0.11, P=0.737). When only fruits that contained 100% seed fill were considered, S: FR ratios were similar between densities at both sites (ABR $F_{1, 446}$=2.65, P=0.105; MOR $F_{1,199}$ =0.00, P=0.986) (Figure 5.6).

The proportion of fruits that were void of seed was very high for ABR (~48%) and MOR (~62%). At ABR, ~35% of fruits overall, contained 100% seed-fill, when compared with ~20% at MOR. Percent seed fill was related to fruit type (i.e. open or autogamous) at ABR (χ^2= 15.57, P=0.004). At MOR, % seed fill categories were similar between autogamous and open fruits (χ^2= 4.01, P=0.405) (Figure 5.7).

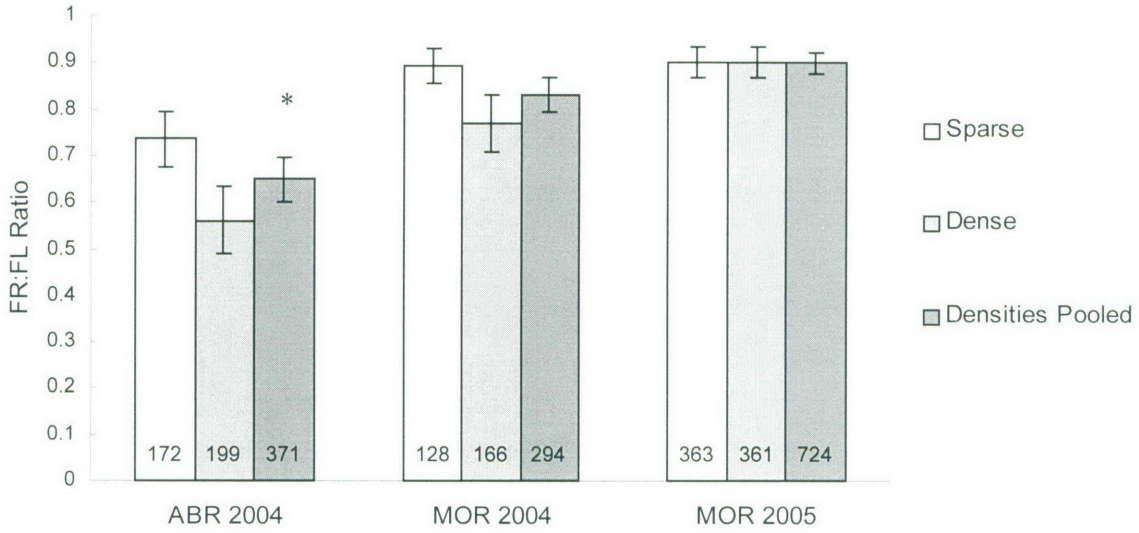

Figure 5.5 Mean FR: FL ratios (±SE) for *T. australe* at ABR for 2004 and MOR for 2004/2005. N (in base of columns)=number of axils scored. * = Ratio (pooled for density) at ABR was significantly lower than at MOR (P<0.05).

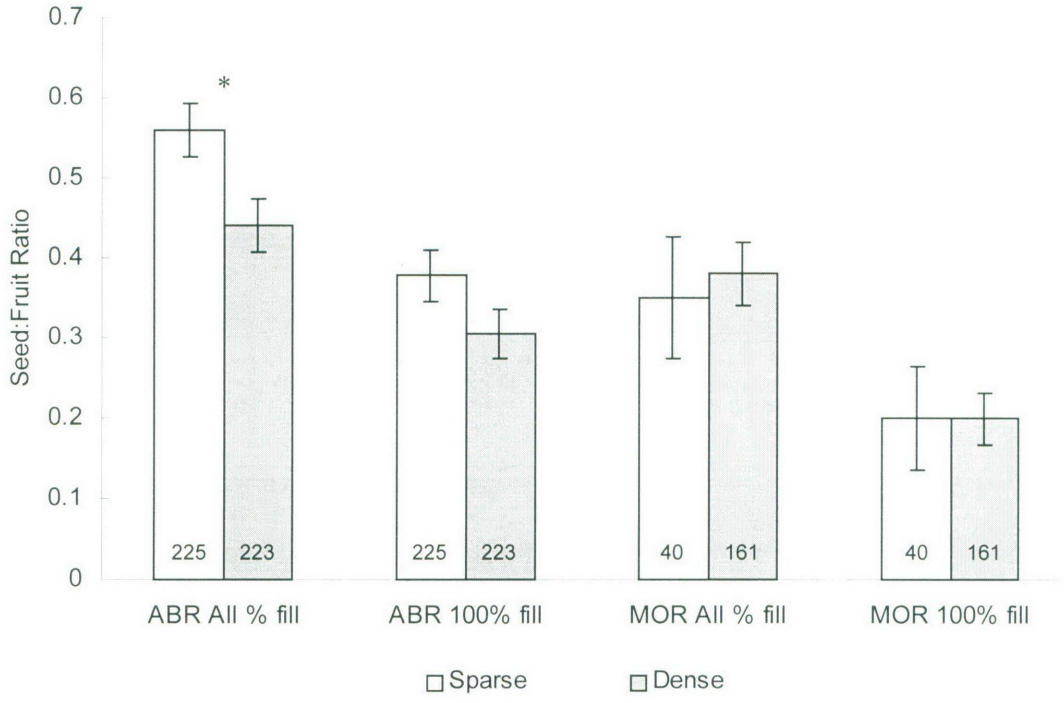

Figure 5.6 Mean S: FR ratios (±SE) for *T. australe* calculated based on i) all fruits that contained seed (25%, 50%, 75% and 100% seed-fill combined) and ii) fruits that contained only 100% seed fill. N (in base of column)=number of fruit. * = Significant between densities (P<0.05).

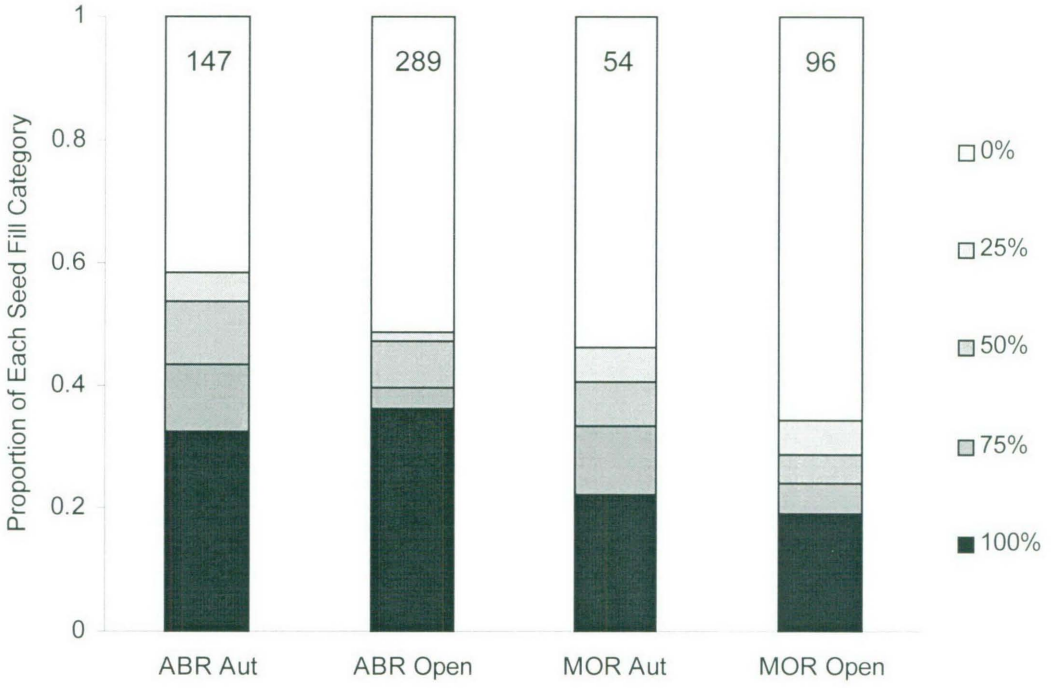

Figure 5.7 Proportion of autogamous and open *T. australe* fruit containing 0, 25, 50, 75 and 100 percent seed-fill at two sites. N (in top of columns)=number of fruit assessed.

5.5.1.2 Wahlenbergia luteola

FR: FL Ratios

In 2004 FR: FL ratios were consistently high across all sites and did not differ between densities within site or for density among sites (Figure 5.8). When FR: FL ratios were pooled for site there was no significant difference between densities (dense mean=0.96±0.03; sparse mean=0.92±0.08) (H=0.258, P=0.611 comparison of medians). In 2005, mean FR: FL ratios were more variable, perhaps due to the implementation of better replication. However, no significant differences were found between densities within sites (POW $F_{1,8}$ =3.68, P=0.091; OAR $F_{1,8}$ =0.70, P=0.427; UNE $F_{1,8}$ =0.97, P=0.354) (Figure 5.8). When sites were pooled for density, UNE (mean=0.71 ± 0.04) had a significantly lower FR: FL than POW (mean=0.87 ± 0.04) and OAR (mean=0.87 ± 0.04) ($F_{2,27}$ =5.74, P=0.008).

S: FR and S: FL ratios

The S: FR ratios for 2004 (actual counts) and 2005 (estimated from bulk weight data) are presented in Figure 5.9. In 2004, with the exception of POW, fruits arising on FPs in dense plots produced more seeds per fruit than sparse, however, this was not significant for any site (POW H=3.401, P=0.65 comparison of medians; OAR $F_{1,11}$ = 3.42, P=0.091 LOG transformed; UNE $F_{1,21}$ = 2.51, P=0.128). There was no difference among sites for dense plots (H=2.032, P=0.362 comparison of medians) but the ratio was variable for sparse plots among sites ($F_{2,35}$ = 6.14, P=0.005) with fruits in POW sparse plots producing significantly more seed than OAR (Figure 5.9). In 2005, all fruits from dense plots produced slightly more seed than those in sparse, but this was never significant (POW $F_{1,8}$ = 1.13, P=0.319; OAR $F_{1,8}$ = 0.10, P=0.765; UNE $F_{1,7}$ = 1.96, P=0.204). Ratios for both densities varied among sites (sparse $F_{2,12}$ = 12.17, P=0.001; dense $F_{2,11}$= 6.40, P=0.014).

In addition to individual seed counts undertaken for the 2004 material, seed weights were used as a surrogate for S: FR ratios for material arising from the 2004 and 2005 seasons. The resulting seed weight: fruit (SW: FR) ratios are presented in Figure 5.10. Mean SW: FR ratios were often higher in dense plots compared with sparse for both years. Seed weight did not vary with density at any site in this year (POW $F_{1,37}$ = 3.69, P=0.063; OAR $F_{1,11}$ = 4.69, P=0.053; UNE $F_{1,21}$ = 1.06, P=0.315). In 2005, dense plots produced significantly higher seed weights per fruit at POW only (POW $F_{1,95}$ = 8.01, P=0.006; OAR $F_{1,98}$ = 0.06, P=0.812; UNE $F_{1,93}$ = 1.70, P=0.195).

Seed to flower ratios (Figure 5.11) were slightly elevated in dense plots compared with sparse for all sites, but this was never significant (POW $F_{1,9}$ = 4.35, P=0.071; OAR $F_{1,9}$ = 0.01, P=0.939; UNE $F_{1,8}$ = 0.23, P=0.643). Neither sparse ($F_{2,14}$ = 3.76, P=0.054) nor dense ($F_{2,13}$ = 2.38, P=0.138) plots differed among sites. When sites were pooled, flowers from sparse plots produced less seed (mean=162.31±13.77) than dense (mean=191.75±19.63), but this was not significant ($F_{1,27}$ = 1.54, P=0.225).

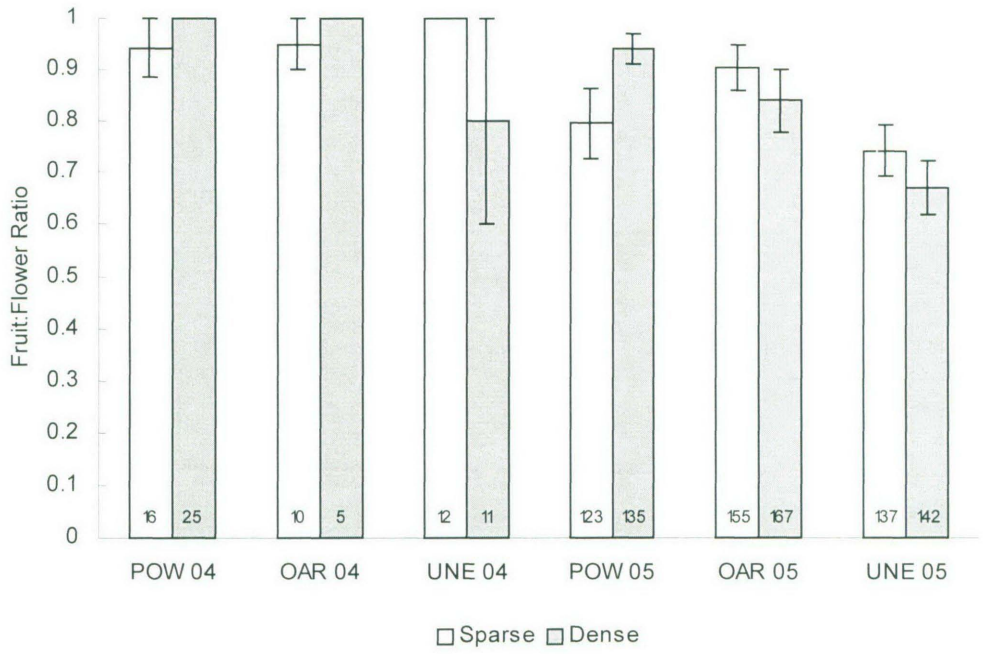

Figure 5.8 Mean FR: FL ratios (±SE) for *W. luteola* at three sites across two years. In 2004, flowers arising on FPs only were assessed. In 2005, to ensure adequate replication, flowers across the plot were assessed. N (in base of column)=number of flowers. All values = 1 for means without ±SE.

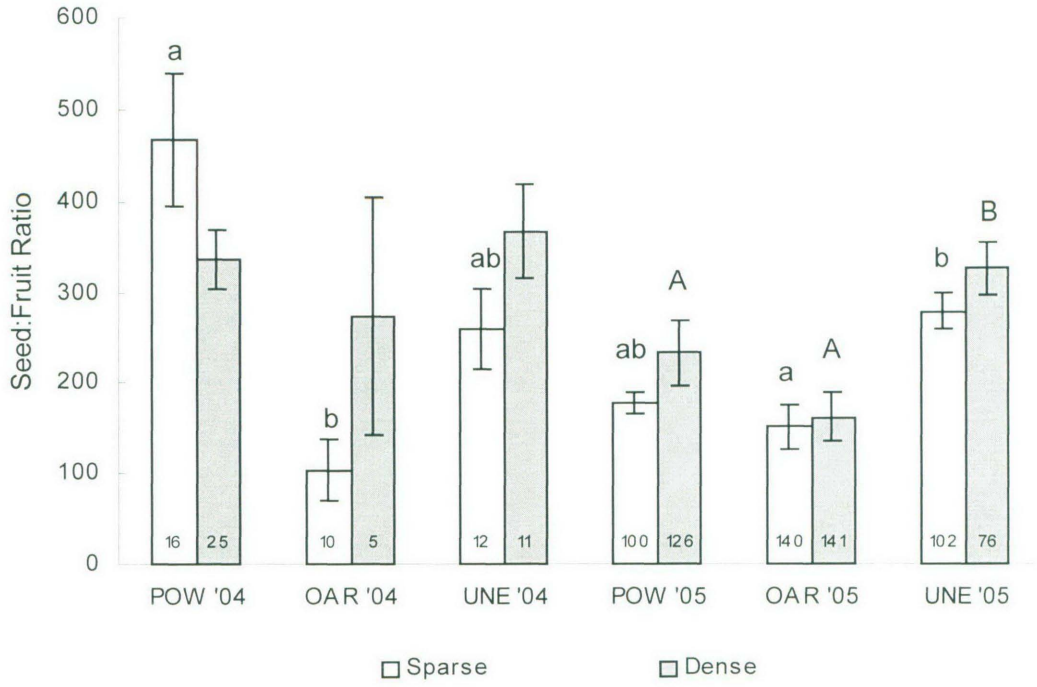

Figure 5.9 Mean number of seeds per fruit (S: FR) (±SE) arising from *W. luteola* focal plants in 2004 and fruits collected across plots in 2005. N (in base of column)=number of fruit. Letters above columns indicate within year differences among sites for density (sparse=lower case, dense=upper case) ($P<0.05$).

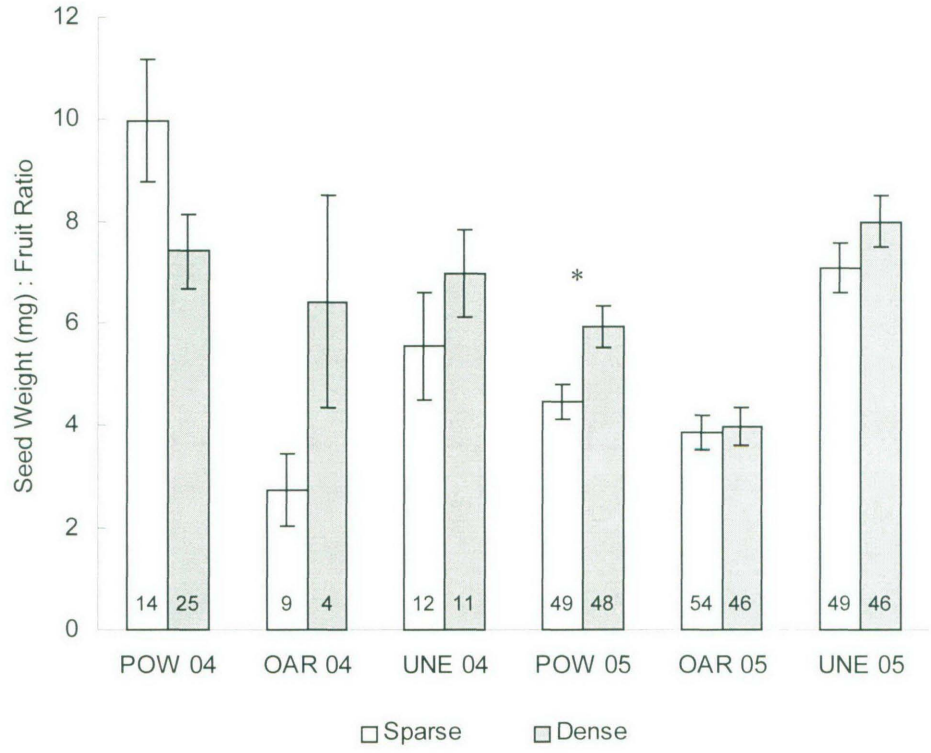

Figure 5.10 Mean weight (mg) of seed content per fruit (SW: FR) (±SE) for *W. luteola* at three sites over two years. N (in base of columns)=number of fruit. * = Significant difference between densities (P<0.05).

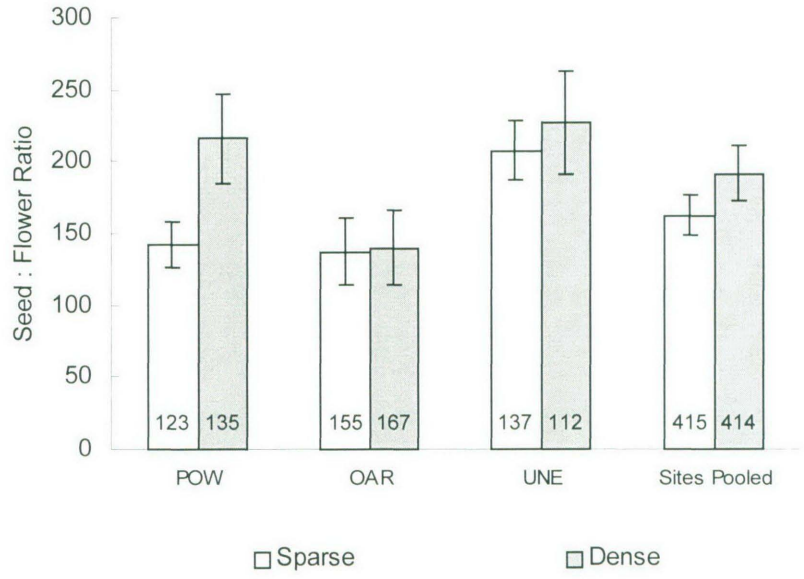

Figure 5.11 Mean S: FL ratio (±SE) for *W. luteola* at three sites in 2005.

Aborted Seed

The level of seed abortion within *W. luteola* fruits was assessed for 2004 material only. The proportion of aborted seed contained within fruits did not differ between densities within site or across sites (Figure 5.12). When densities were pooled among sites, abortion rates varied significantly; OAR (30%) > POW (18%) > UNE (11%), with UNE having significantly lower levels than OAR and POW ($F_{2,72}$ =9.41 P=0.000 LOG transformed).

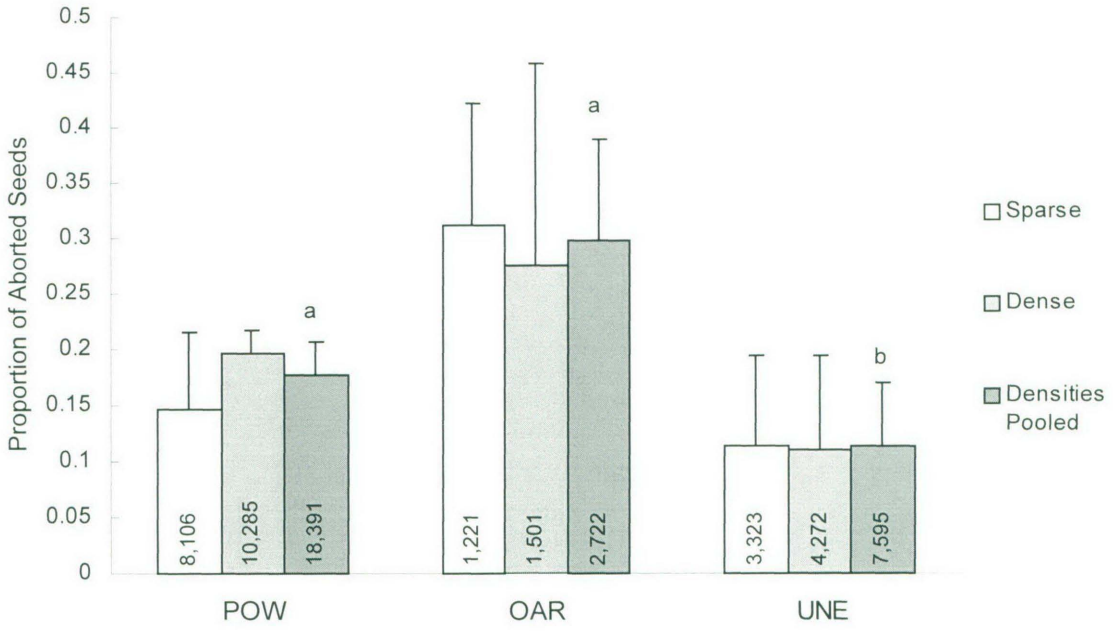

Figure 5.12 Mean proportion of seeds (±SE) that were aborted within *W. luteola* fruits at three sites N (in base of columns)=number of seeds and two densities. Letters above columns denote significant differences among sites for densities pooled (P<0.05).

Pollen Limitation

To investigate the level to which pollen may have been limiting seed production in sparse and dense plots, S: FR ratios were compared with the S: FR ratios that resulted from the supplemented outcross treatment imposed for the breeding system experiment (Chapter 3). Compared with flowers that received outcross pollen supplementation, flowers in sparse plots produced around half as many seeds whereas in dense plots, seed production was only reduced by around one third (Table 5.2) in controls. This suggests that pollen quantity was more limiting to seed production in sparse plots compared with dense plots.

Table 5.2 Mean S: FR ratio for open pollination *W. luteola* flowers in sparse and dense plots at three sites and two years compared with S: FR ratio arising from flowers supplemented with outcross pollen. * Value not included in overall mean % reduction calculation.

	Mean S: FR ± SE (Mean % Reduction Compared with Supplemental Cross)						
	POW		MOR		OAR		Mean % Reduction
	'04	'05	'04	'05	'04	'05	
Supplemental Cross Treatment	455.7±40.6		404.8±30.7		409.8±32.7		-
Sparse Plots	467.3±71.8 (+1%)*	177.8±11.4 (-61%)	103.7±33.7 (-75%)	151.0±24.0 (-63%)	259.7±44.1 (-37%)	278.0±19.9 (-32%)	54%
Dense Plots	335.2±32.5 (-27%)	232.4±51.5 (-49%)	273.8±30.4 (-32%)	162.0±26.2 (-60%)	366.6±51.5 (-11%)	325.8±29.0 (-21%)	33%

5.5.1.3 Dillwynia sieberi

FR: FL Ratio

Fruit: flower ratio is based on all fruits, whether predated or containing aborted or healthy seed. The ratio was consistent among sites for sparse plots (2004 $F_{2,69}$ = 1.31, P=0.277; 2005 $F_{2,57}$ = 1.79, P=0.176) and dense plots (2004 H=0.342, P=0.843 comparison of medians; 2005 $F_{2,53}$ = 1.45 P=0.243), and data were therefore pooled. Overall, FR: FL were significantly higher for dense than for sparse plots in both years (2004 $F_{1,139}$=4.32, P=0.0395; 2005 $F_{1,114}$=6.80, P=0.010) (Figure 5.13). Between year differences were not evident for sparse plots ($F_{1,129}$=0.01, P=0.927) and only marginally significant for dense plots (H=3.94, P=0.047 comparison of medians). Interestingly, dense plots produce higher FR: FL ratios than do sparse. However, a true level of reproductive output can only be accurately measured by taking into account the level of seed success and loss due to predation and abortion. Therefore, seed to fruit (S: FR) and seed: flower (S: FL) ratios were investigated.

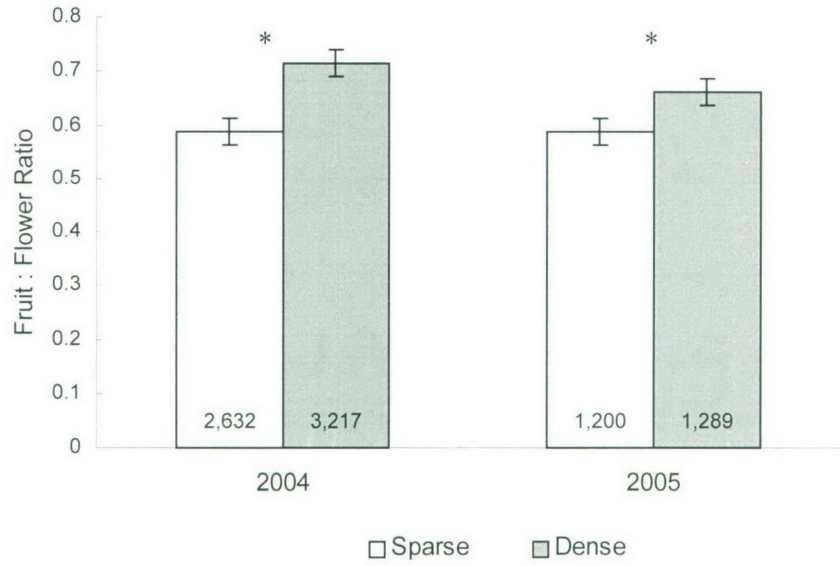

Figure 5.13 Mean FR: FL ratios within sparse and dense plots for *D. sieberi* in 2004 and 2005. N (in base of columns)=number of flowers.
* Above columns=significant differences between densities (P<0.05).

S: FR and S: FL Ratios

Two S: FR ratios were calculated. The first excluded fruits that had been predated, thus giving an indication of seed set in the absence of seed feeders (Figure 5.14a). The second included the predated fruits in the counts, giving the realised seed production per fruit after the effects of predation (Figure 5.14b). In 2004, S: FR (in the absence of predation) was lower in dense plots for two sites, but density was only significant at OAR (POW $F_{1,35}$=0.45, P=0.506; MOR H=0.077, P=0.782; OAR $F_{1,41}$=7.96, P=0.007). In 2005, the trend was for S: FR ratios to be somewhat higher in dense plots, but density was never significant (POW $F_{1,24}$=1.76, P=0.197; MOR $F_{1,24}$=0.52, P=0.476; OAR $F_{1,18}$=0.00, P=0.983) nor was density significant when sites were pooled ($F_{1,70}$=1.23, P=0.272).

When predation was factored into the S: FR ratio (Figure 5.14b), the result was far more variable than observed in its absence and overall seed production was substantially reduced in most cases. There was no significant difference between densities at POW ($F_{1,37}$=0.03, P=0.859) or MOR ($F_{1,40}$=0.28, P=0.603) but seed production was significantly lower in sparse plots at OAR ($F_{1,41}$=6.86, P=0.0.012). S: FR ratios varied for both sparse ($F_{2,59}$=7.36, P=0.001) and dense ($F_{2,59}$=4.64, P=0.013) plots among sites in this year. In 2005, densities were similar both within sites (POW $F_{1,32}$=0.01, P=0.927; MOR $F_{1,34}$=2.15, P=0.152;

OAR $F_{1,33}$=0.89, P=0.352) and among sites (sparse $F_{2,53}$=0.49, P=0.614; dense $F_{2,46}$=0.78, P=0.465). These results, when compared with the FR: FL results, clearly indicate the need to incorporate seed production before making assumptions about reproductive output based on FR: FL ratios.

There was similar variability in S: FL ratios (Figure 5.15). No differences between densities was revealed for any site in 2004 (POW $F_{1,45}$=0.35, P=0.555; MOR $F_{1,44}$=0.66, P=0.420; OAR $F_{1,47}$=1.49, P=0.228) or 2005 (POW $F_{1,39}$=0.16, P=0.688; MOR $F_{1,63}$=0.00 P=0.959; OAR $F_{1,34}$=1.49, P=0.458). S: FL ratios in sparse plots varied significantly among sites in 2004 ($F_{2,71}$=4.09, P=0.021) but not in 2005 ($F_{2,61}$=1.76, P=0.181) and dense plots behaved similarly among sites in 2004 ($F_{2,66}$=2.45, P=0.094) and 2005 ($F_{2,53}$=0.05, P=0.949).

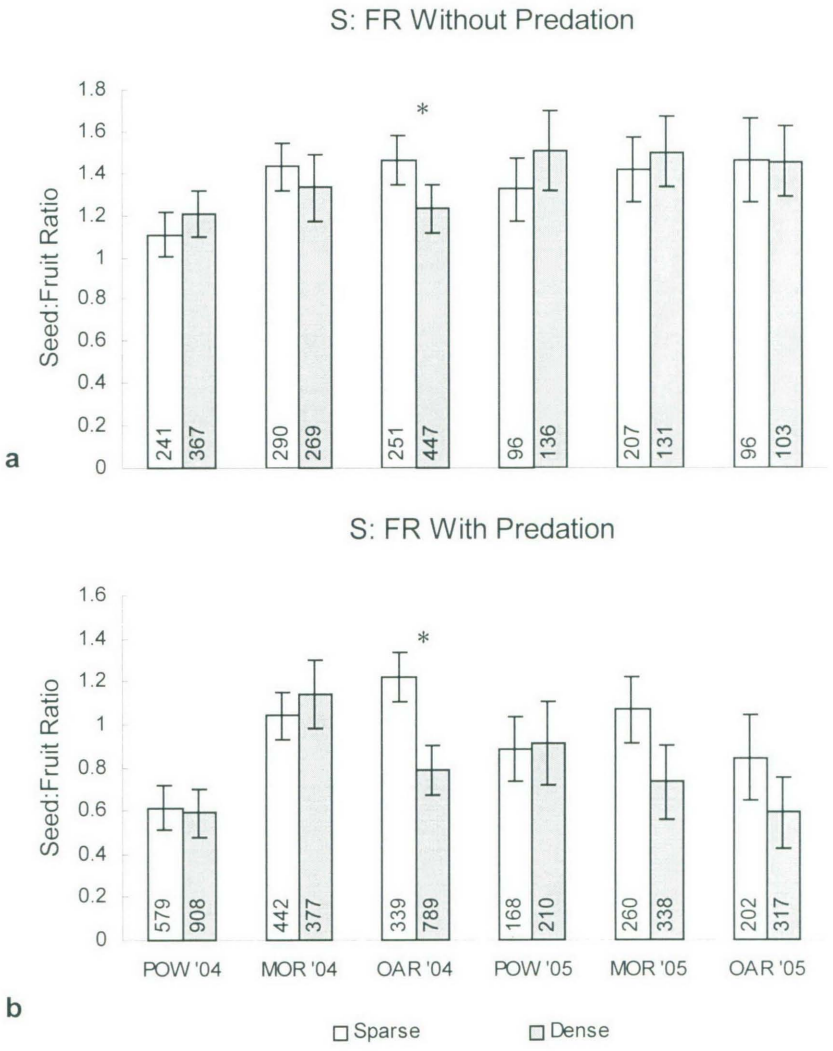

Figure 5.14 Seed to fruit ratios (±SE) for *D. sieberi* at three sites and for two years **a)** seed set without predation and **b)** seed set including predation. N (in base of columns)=number of fruit. * Above column pairs=significant within site density differences (P<0.05).

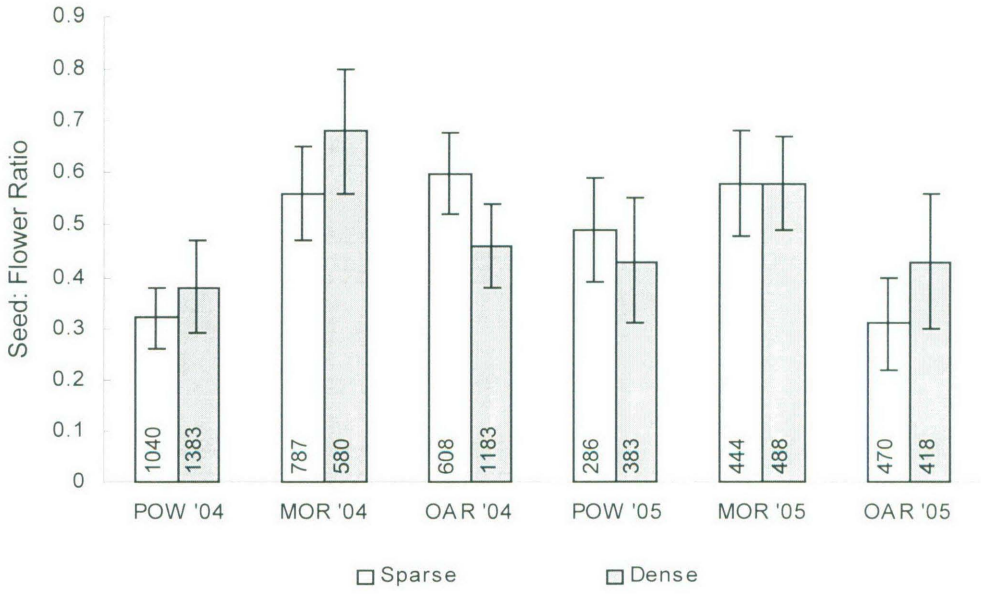

Figure 5.15 Mean seed to flower ratio (S: FL) (±SE) for *D. sieberi* across three sites and two densities and years. N (in base of columns)=number of flowers. No significant differences were detected.

Proportion of Aborted Fruit

The proportion of fruits containing aborted seed was variable among sites in 2004. There was no difference among sites for sparse plots ($F_{2,23}$ = 0.54, P = 0.589 LOG transformed) however for dense plots, there was a significantly higher proportion at MOR compared to OAR ($F_{2,32}$ = 3.66, P = 0.037 LOG transformed). Between density within site differences were non-significant (POW ($F_{1,45}$ = 0.11, P = 0.746; MOR $F_{1,21}$ = 2.65, P = 0.118 LOG transformed; OAR $F_{1,16}$ = 0.62, P = 0.442 LOG transformed) (Figure 5.16). In 2005, there was no difference among sites for proportion of fruit aborted in sparse ($F_{2,56}$ = 0.06, P = 0.94) or in dense plots ($F_{2,51}$ =1.14, P= 0.33). Apart from MOR in 2004, density did not appear to have an effect on the incidence of aborted fruit.

Incidence of 1- and 2-Seeded Fruits

Dillwynia sieberi flowers contain two ovules and produce 1- or 2-seeded fruit. Since a low incidence of 2-seeded fruit could indicate pollen limitation, the relative proportion of 1- and 2-seeded fruit between densities was assessed (Figure 5.17). Proportions were calculated based on 1,273 undehisced fruits from stems bagged (10 FPs per site) to assess predation

levels, where proportions were averaged for plot density. There were no differences among sites for the proportion of 1-seeded or 2-seeded fruits (sparse $F_{2,11}$ =0.57, P= 0.882; dense $F_{2,8}$ =0.51, P= 0.623) and sites were pooled. There was a higher proportion of 1-seeded fruits produced in sparse plots ($F_{1,23}$ =4.37, P= 0.048) than in dense plots (Figure 5.17).

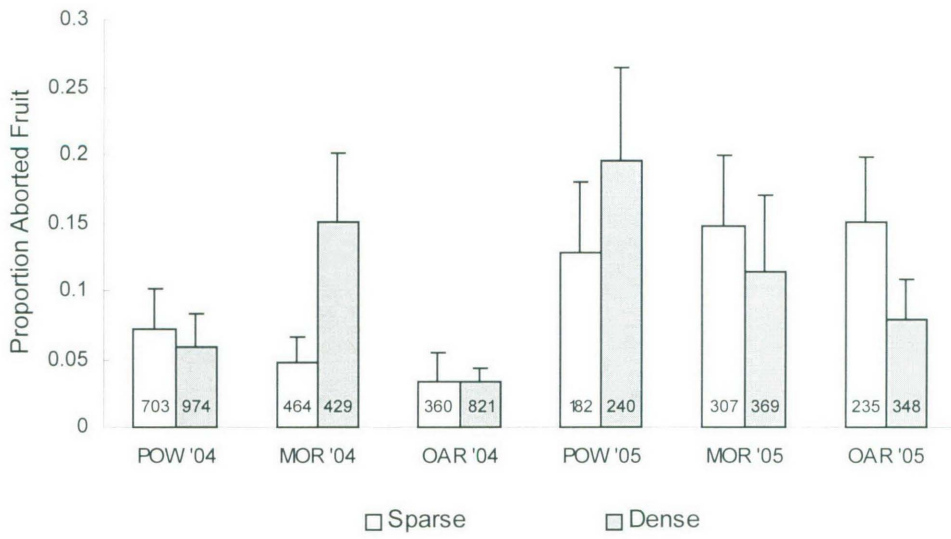

Figure 5.16 Mean proportion of fruit (±SE) that contained aborted seed for *D. sieberi* in sparse and dense plots in 2004 and 2005 N (in base of columns)=number of fruit.

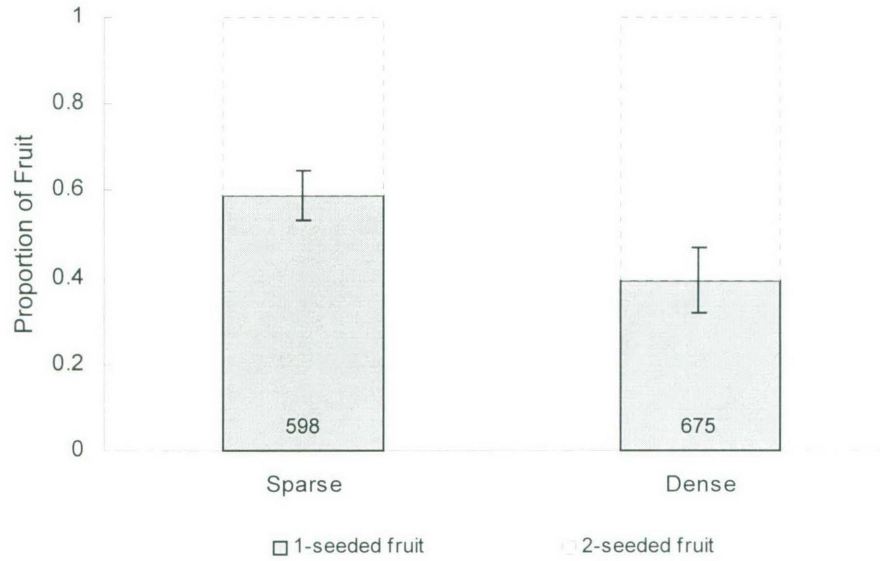

Figure 5.17 Mean proportion (±SE) of 1-seeded fruits produced in sparse and dense *D. sieberi* plots in 2005. N (in base of columns)=number of fruit.

Fruit Predation

Predation data were collected from three sources; stems bagged for FR: FL ratios in 2004, stems bagged specifically to assess predation in 2004 and stems bagged for FR: FL ratios in 2005 (labelled '04a, '04b and '05 respectively in Figure 5.18). Predation on the 2004 FR: FL stems was variable within sparse ($F_{2,69}$ =7.36, P= 0.001 SQRT transformed) and dense ($F_{2,66}$ =5.71, P= 0.005) plots among sites, with the POW site experiencing the highest levels of predation overall. Density did not appear to strongly influence predation (POW $F_{1,38}$ =0.11, P=0.747; MOR $F_{1,27}$ =0.12, P=0.729) and was only significant for OAR ($F_{1,46}$ =6.14, P= 0.017). For the 2004 predation stems, there was no significant difference among sites for density (sparse $F_{2,12}$ =0.24, P=0.791; dense $F_{2,10}$ =4.09, P=0.05) nor between densities when pooled for site ($F_{1,26}$ =0.04, P=0.848). In 2005, no significant difference was revealed for the proportion of fruit predated among sites for sparse ($F_{2,55}$ =1.16, P= 0.321) or for dense ($F_{2,51}$ =2.26, P= 0.115), and density was only significant for MOR (POW $F_{1,39}$ =0.07 P= 0.798; MOR $F_{1,37}$ =6.10 P= 0.018; OAR $F_{1,34}$ =2.24 P= 0.144). Overall, there was no clear discernable trend for predation rates relating to site or density. A multifactor ANOVA confirmed this, but revealed some significant interactions between sites and plot type and site and collection (Table 5.3).

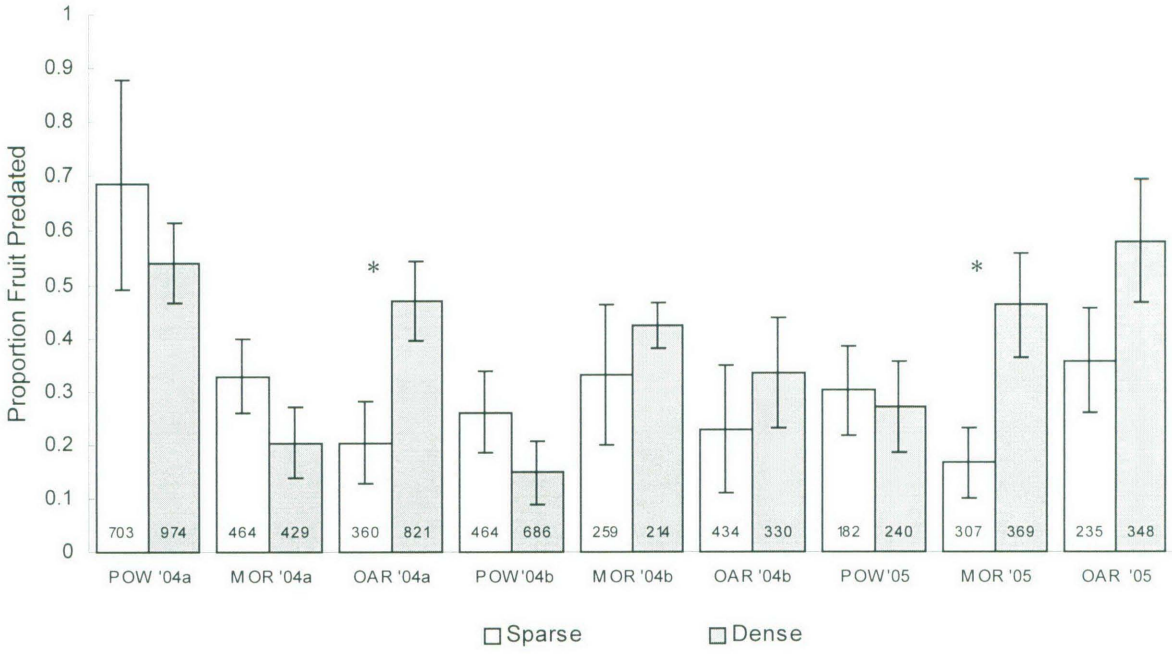

Figure 5.18 Mean proportion (±SE) of *D. sieberi* fruits predated across three sites and two densities for 2004 (two sampling events '04a and '04b) and 2005. N (in base of columns)=number of fruit. *Above columns=Significant difference between densities (P<0.05).

Table 5.3 Multifactor ANOVA of proportion of *D. sieberi* fruit predated in 2004 (two sampling events) and 2005 (one sampling event).

	Proportion of *D. sieberi* Fruits Predated		
Source	*d.f*	*F*	*P*
Site	2	0.21	0.808
Density	1	0.89	0.346
Stem ID	2	0.93	0.394
Site x Density	2	3.25	0.040
Site x Sampling Event	4	3.78	0.005
Density x Sampling Event	2	1.09	0.336
Residual	271		
Total	284		

5.5.2 Relationship Between Visitation and Reproduction

5.5.2.1 Wahlenbergia luteola

Regression analysis revealed no relationship between visitation rates to *W. luteola* plots and S: FR ratios for either 2004 (r=0.11, $F_{1,21}$ = 0.29, P=0.599) or 2005 (r=0.183, $F_{1,21}$ = 0.73, P=0.402).

5.5.2.2 Dillwynia sieberi

Simple linear regression revealed a significant positive relationship between visitation rate to FPs and FR: FL ratios in 2004 (r=0.433, $F_{1,19}$ = 4.39, P=0.049); there was no relationship in 2005 (r=0.202, $F_{1,16}$ = 0.69, P=0.421); (Figure 5.19). No relationship was found between visitation rate and S: FR ratio in either year (2004 r=-0.0370, $F_{1,19}$ = 3.01, P=0.099; 2005 r=-0.402, $F_{1,16}$ = 3.09, P=0.098).

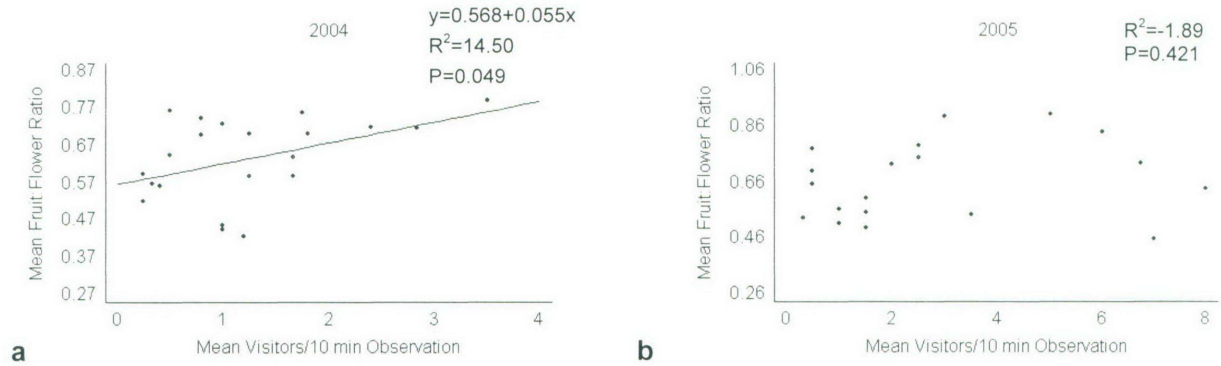

Figure 5.19 The relationship between mean visitation rate and mean FR: FL ratio for *D. sieberi* in **a)** 2004 and **b)** 2005

5.5.3 Results Summary

An overview of results for this chapter is given in Table 5.4. The results clearly show that it is important to utilise multiple study sites, as variability among sites is common. In some instances, the overall trend indicated that reproductive output was positively influenced by local density, but this was only significant for the self-incompatible *D. sieberi* FR: FL ratios. Although FR: FL ratios are an important measure of reproductive output, they are not always the most useful. For instance, seed production varies significantly per fruit in *W. luteola,* and S: FR ratios were better indicators of reproductive success for this species.

Table 5.4 Summary of findings relating to reproductive output for the three study species.

Trend = was there an overall trend for a particular density? ; ↑ = Tended to be higher overall, ↓ = Tended to be lower overall; * = Difference was statistically significant; S= sparse D = dense;✓=Yes, X=No; Site = were there significant within year differences among sites? (S & D indicate which density varied); Relationship = was a relationship detected? ; - = no data

Species	Year	Variable Measured							
		FR: FL		S: FR		S: FL		Vis. vs FR: FL	Vis. vs S: FR
		Trend	Site	Trend	Site	Trend	Site	Relationship	Relationship
T. australe	'04	↓ D	✓	-	-	-	-	-	-
	'05	X	-	X	✓	-	-	-	-
W. luteola	'04	X	X	↑ D	✓ S	-	-	X	X
	'05	X	X	↑ D	✓ S & D	↑ D	X	X	X (but pollen limitation ↓ D)
D. sieberi	'04	↑ D *	X	↓ D	✓ S	X	✓ S	✓	X
	'05	↑ D *	X	↑ D	X	X	X	X	X

5.5 Discussion

The results did not offer consistent statistically significant indications that reproduction was density-dependant for any of the study species, however this is not uncommon, especially in studies undertaken in natural systems (e.g. Aizen 1997). Nonetheless, there were several occasions where results were suggestive of density effects, at least for *W. luteola* and *D. sieberi*. The tendency for FR: FL, S: FR and S: FL ratios to yield conflicting results makes the ratio an important component to consider when investigating reproductive effort and interpreting results. Clearly, the relative utility of these ratios is dependent on the reproductive ecology of individual species and on external environmental influences, such as predation. Assessment of reproductive responses may need to incorporate a combination of these variables to adequately evaluate a given situation.

Although there was some evidence to suggest that fruit and seed set was independent of density, the data acquired for *T. australe* was inadequate to comprehensively assess density responses within and among sites. Fruit set was reasonably high and ranged between ~70% and ~90%. Based on these figures alone, it would be reasonable to assume that the populations are reproductively sound. However, an interesting aspect of *T. australe's* seed production was revealed i.e. the prevalence of fruits that were void of, or only partially filled with, seed. Partial seed-fill is likely to represent reduced fitness and as discussed in Chapter 6, a direct relationship was found between partial seed-fill and reduced viability; as Levin (1984) points out the seed development stage is an important phase in the life history of plants for identifying the effects of inbreeding. Furthermore, fruits that were completely filled (100% seed fill) were seldom encountered at either site (~35% at most) and fruits that were completely void of seed comprised up to ~60% of fruit produced. Such high levels of seed abortion indicate a high degree of inbreeding depression in these populations. Fruit that resulted from both autogamy and from flowers that were left open to potential pollinators (of which none were observed, see Chapter 4) produced similar levels of partially filled fruit. However, it is not unusual for selfed seed production to be reduced compared with seed resulting from outcrossing. For instance Schemske (1983) found markedly reduced seed set in selfed progeny in three *Costus* spp. (Zingiberaceae) compared with their outcrossed counterparts. It seems reasonable to expect that if the *T. australe* flowers that were left to open pollination had produced fruit xenogamously, then some indication of increased seed output or

fitness might be observed. The above results may represent further evidence that most (if not all) of the fruit produced by *T. australe* is autogamous within these populations. However, this can only be thoroughly investigated by undertaking further breeding system work in conjunction with exploration of the genetic constitution of the populations.

Wahlenbergia luteola flowers can often produce fruits that appear to be healthy, but when opened contain a full complement of aborted seed or at best, very few apparently healthy seeds. Therefore, using FR: FL ratios in isolation to assess reproductive output in this species is far from ideal. It appears that in order for FR: FL ratios to provide useful information about reproduction within the population, relatively large sample sizes are required from a broad range of individuals (compare 2004 and 2005 results, Figure 5.8). The S: FR ratios for this species were far more informative. There was some indication (although not significant) that seed set in dense arrays was higher than that in sparse, which would be expected considering the significantly higher visitation rates to dense plots. Although no direct relationship could be found between visitation rate and seed set for this species, there were still some indications that seed set may be pollinator limited (such as the tendency for net seed production (S: FL) to be higher in dense plots).

Breeding system work on *W. luteola* (Chapter 3) indicated slightly lower seed production under open pollination when compared with flowers supplemented with cross pollen. Although these treatments weren't significantly different, pollen limitation levels may vary slightly among these populations, since ovule fertilisation under supplementation was estimated to be ~90% for two sites and approached 100% at the third (extrapolated by comparing the mean number of ovules/flower and mean S: FR production under supplementation (Chapter 3)). Moreover, compared with seed production arising from outcross pollen supplementation, seed set was reduced overall by around 50% in sparse plots and 30% dense plots indicating a differential between densities in pollen limitation (Table 5.2). Therefore, pollen quantity (moderated by reduced visitation) rather than quality may be limiting seed production in sparse individuals. Furthermore, since *W. luteola* is protandrous, the opportunities for self-pollen receipt (which results in highly reduced seed set-Chapter 4) are considerably diminished equally for both sparse and dense flowers (however, self-pollen removal may be more extensive in dense plots due to higher visitation rates there than in sparse plots).

Since *W. luteola* is a facultative outcrosser and density clearly modulates visitation, these results suggest that in a system where pollination is limiting, patches of low local density could suffer reductions in xenogamous seed set. *Wahlenbergia luteola* is self-compatible and a lack of visitation could promote the production of seed via autogamy (which is likely to be of lower quality than xenogamous seed). The extent to which automatic selfing occurs in *W. luteola*, appears to vary among sites (Chapter 3) and enclosing the flowers in bags may have promoted it to some extent, by forcing the stigmas to come into close contact with pollen. However, autogamy is common in several *Wahlenbergia* spp. on Robinson Crusoe Island (Chile) where pollinators are extremely scarce (5 visitors observed in 50 hours of observation) (Anderson *et al.* 2000). Mechanisms promoting autogamy on the island include; the recurvature of stigmatic lobes onto the style (which pick up self-pollen remaining on the style) and utilising wind to bring the stigma into contact with the inside of the corolla where pollen has been deposited during the male phase. For all of these species, pollen and ovule numbers were similar to those observed for *W. luteola*, yet seed production from open pollination on the island was very low (the highest individual S: FR ratio was 188 and most fruits produced <50 seeds compared with the several hundreds of seeds per fruit often observed in this study) (Anderson *et al.* 2000). The situation on Robinson Crusoe Island clearly illustrates the extent to which the loss of pollinators can reduce seed set, but also illustrates the capacity of self-compatible species to persist under less than favourable circumstances.

There was a strong indication that reproduction was positively influenced by local density for *D. sieberi* as FR: FL ratios were significantly higher in dense plots for both of the study years. There was also a positive relationship between visitation rate and FR: FL ratio, (but for 2004 only). This outcome is expected in light of *D. sieberi's* strong self-incompatibility and pollinator dependence; similar patterns have been observed in other self-incompatible species (e.g. Kunin 1992, 1997a; Metcalfe & Kunin 2005). However, the significant density effects in this study did not extend to S: FR ratios. Again, this highlights the importance of utilising multiple ratios that span all levels of reproduction to assess fecundity. For instance, a converse situation was found by Aguilar & Galetto (2004) where seed production was negatively correlated with fragmentation, but there was no effect on fruit production for *Cestrum parqui* (Solanaceae). Nevertheless, there were some interesting

patterns regarding seed production, which are likely due to the density-dependent foraging behaviour of *D. sieberi* visitors.

The flowers of *D. sieberi* possess two ovules, and dense plots produced significantly more two-seeded fruits than did sparse. One would expect that the delivery of pollen to plants in dense plots would be superior (in terms of both quality and quantity) compared with sparse, due to the proximity of conspecifics, and that this would facilitate more frequent fertilisation of both ovules. The tendency for 1-seeded fruit to be produced in sparse plots is therefore probably due to reduced visitation and/or to increased intra-plant pollen movement; both HBs and NBs were found to preferentially visit more flowers on sparse *D. sieberi* individuals compared with dense (Chapter 4). Furthermore, it appears that HBs may be inefficient pollinators. Gross (2001) found that fruit set only occurred in 14.5% of flowers allowed a single visit by a HB, whereas FR: FL ratios for flowers left to open pollination were ~70%. This is a substantial difference and suggests that optimal fruit production requires more than one visit by a HB and that the number of visitations to an individual flower is an important restraint to fruit production; it stands to reason that seed production may be similarly affected. In the absence of predation, S: FR ratios in this study were often higher (although not significantly so) in dense plots and this is likely due to the higher incidence of two-seeded fruits which may have been facilitated by individual flowers receiving a greater number of visitors (pollen quantity) which were also carrying pollen loads comprising more outcross pollen (pollen quality).

Gross (2001) found no evidence that *D sieberi* was pollen limited, but fruit set was never 100% after supplementing with outcross pollen, therefore other factors are probably operating to limit fruit set (and subsequent seed set) in those populations; this could include resource limitation (Vaughton 1991) and/or underlying genetic influences. Furthermore, the effect of seed predation on net reproductive output for *D. sieberi* was substantial, but there was no evidence that it was density dependent. Thus, although visitation is clearly important to *D. sieberi* reproduction and there is evidence that fecundity is shaped by visitor responses to density, net reproductive success for this species (as reflected in S: FL ratios) is ultimately determined by other factors.

Thus far, there is little indication that reproduction in *T. australe* is density dependent and although self-compatibility affords it reproductive assurance in the absence of pollinators,

the results suggest that the amount of seed produced (and potentially its quality) is negatively affected by inbreeding. There is some evidence that visitation to both *W. luteola* and *D. sieberi* is density dependent and that both visitation rate and visitor behaviour are influential to their reproduction (as moderated by their respective breeding systems). The following chapter investigates offspring fitness for these species, to determine the extent to which these processes may influence subsequent generations. Male fitness (pollen tube growth) is also assessed.