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REPRODUCTIVE ECOLOGY OF *RANDIA DUMETORUM*: FACTORS  
AFFECTING THE HIGH FLOWER TO FRUIT RATIO

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A thesis submitted in partial fulfilment of the requirements  
for the BS-MS dual degree programme in IISER Pune

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## Certificate

This is to certify that this dissertation entitled 'Reproductive Ecology of *Randia dumetorum*: Factors affecting the high flower to fruit ratio' towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research (IISER), Pune represents original research carried out by Sameer Parihar at IISER Pune under the supervision of Dr. Deepak Barua, Assistant Professor, Biology Division, IISER Pune during the academic year 2011-2012.

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## Declaration

I hereby declare that the matter embodied in the thesis entitled 'Reproductive Ecology of *Randia dumetorum*: Factors affecting the high flower to fruit ratio' are the results of the investigations carried out by me at the Biology Division, IISER Pune under the supervision of Dr. Deepak Barua, Assistant Professor, Biology Division, IISER Pune and the same has not been submitted elsewhere for any other degree.

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## Abstract

In this study, we examined the reproductive ecology of *Randia dumetorum* at Bhimashankar, which constitutes the Northern limit of Western Ghats. We investigated the various factors which affect the fruit set of the plant, namely resource availability, pollination, phenology, predation etc. In *Randia*, stored resources were observed to have an effect on fruit set of *Randia*, high fruit number in the previous year resulted in low fruit number the next year and the trees which had low fruiting the previous year were bearing high number of fruits the next year. Resource allocation was also found to be non-uniform, there was branch-specificity attached to trees with higher fruit number. Flowering phenology was observed to have no effect on flower number but late onset of flowering and shorter flowering duration resulted in high fruit number. Another factor, predation, was checked and found that there are signs of at least two pre-dispersal predators. One of them identified as *Virachola perse ghela*. Pre-dispersal predation did not depend on flowering start date but longer flowering duration lead to less predation. Also, the proportion of infested fruits decreased with the increase in total fruits, possibly leading to predator satiation.

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## **INTRODUCTION**

Fruit to flower ratio in a plant has baffled the researchers for a long time. A huge amount of research is done to figure out what drives the plants to make abundant amount of flowers but develop only a small fraction of it to fruits.

The studies have shown that the plant phenology ( Brody, 1997; Elzinga et al., 2007; Kudo & Suzuki, 2002), number of flowers (Delph & Sutherland, 1984; Sabat & Ackerman, 1996; Stephenson, 1984; Sutherland, 1986a, 1986b), attractiveness of plant (M Burd & Callahan, 2000; Robert Wyatt, 1981), pollinator density (Ashman et al., 2004; M Burd & Callahan, 2000; R. Wyatt, 1982), plant density (Wilcock & Neiland, 2002; Robert Wyatt, 1981), fragmented landscapes (A. T.-lynn Ashman et al., 2004; Knight et al., 2005), spatial organisation (Barrett, 1998; Diggle, 1995; Obeso, 2002) and temporal variation (Barrett, 1998; Diggle, 1995; R. Wyatt, 1982) of flowers within a plant, breeding system (hermaphrodite, monoecious, dioecious etc.)(Delph & Sutherland, 1984; Knight et al., 2005; Primack, 1987; Sutherland, 1986a, 1986b), mating system (self/cross pollen)(Abe, 2001; Barrett, 1998; Delph & Sutherland, 1984; Knight et al., 2005; Primack, 1987; Sutherland, 1986a, 1986b), resource allocation (Knight et al., 2005; Stephenson, 1984; Sutherland, 1986a, 1986b; Robert Wyatt, 1981), leaf herbivory (Brody, 1997; Ehrlén, 1996; Stephenson, 1984)and predation (Andersen, 1989; Ehrlén, 1996; Hulme, 1994; Jansen, 1971; A. T. Moles, Warton, & Westoby, 2003; Parachnowitsch & Christina M. Caruso, 2008; Zimmerman, 1980) affects the no. of fruits the plant produces.

When studied the relationships between some of these factors (Fig. 1). Most of the factors were interrelated and boiled down to two major factors – resource limitation and pollen limitation.

### **Resource Availability**

Resources are the basic requirement for the plants. Resources available are used by the plant for growth, reproduction, maintenance, defence and storage (Abe, 2001; Stephenson, 1981, 1984; Sutherland, 1986b; Wesselingh, 2007). For growth purposes, plants increase the number of roots and shoots, number of leaves and its size , or allo-

cate resources for storage for future growth (Stephenson, 1981; Sutherland, 1986b; Wesselingh, 2007). For reproductive purpose, the allocation of resources goes towards the number of flowers, the nectar content of the flowers, the ovule number in flowers and size of the fruit (M Burd & Callahan, 2000; M. Morgan, 1993; Stephenson, 1981; Sutherland, 1986b; Wesselingh, 2007). Thus in situations where resource are limiting it becomes a very important factor in determining the percentage fruit set.

Resources can be limiting when plant density (Obeso, 2002; Wilcock & Neiland, 2002) or leaf herbivory (Brody, 1997; Crawley, 1989; Stephenson, 1981, 1984) increases. For individual flowers or fruits, resources can be limiting when there are a large number of fertilized flowers and fruits (R. Wyatt, 1982) or when the flower is at the terminal end of a branch (i.e. the distance between the source and consumer is high) (Diggle, 1995).

Resource limitation can be created if some of its flowers get pollinated. When a flower gets fertilized, it requires a lot of resource for growth of a fruit. Hence, it becomes a sink for the resources present in the plant. If the resources available are fixed in quantity, it can create resource limitation for the rest of the plant (Diggle, 1995).

## **Pollen limitation**

In past, resources were given greater importance for affecting fruit set but in the last 20-30 years studies have identified pollen limitation to be a major player in determining fruit set (Aizen & Harder, 2007; A. T.-lynn Ashman et al., 2004; Casper & Nisenbaum, 1993; Delph & Sutherland, 1984; Sutherland, 1986a, 1986b; Wesselingh, 2007). Pollen limitation can occur due to many different factors:

1. **Pollen quantity:** The amount of pollen deposited on a flower is insufficient to induce fertilization. It can happen due to many factors such as-
  - a) Pollinator density: when the number of species specific pollinators available are low in number (M Burd & Callahan, 2000; Martin Burd, 1994).
  - b) Pollinator competition: when there is a generalist pollinator for many species (Berry & Calvo, 1991; Cariveau et al., 2004; Elzinga et al., 2007).
  - c) Flowering time: If the different individuals of the same cross pollinating species have a staggered flowering time, then the amount of pollen avail-

able for pollination at a given time will be less (A. T.-lynn Ashman et al., 2004; Wesselingh, 2007).

2. **Pollen quality:** The quality of pollen received by the flower is low, i.e. low fitness or the pollen of the same plant is deposited on itself for cross pollinating species (Aizen & Harder, 2007; M Burd & Callahan, 2000; Knight et al., 2005).

It can happen due to various reasons like large number of nectar rich flowers, fragmented landscape, low plant density etc. which will result in the pollinator staying longer on the same plant as the benefits will be greater to stay than to move to a second plant (Barrett, 1998; Delph & Sutherland, 1984).

3. **Mating System:** The mating system of a species is also a factor determining the extent of pollen limitation. For a cross pollinating species the chances of pollen limitation is higher than self pollinating or a geitonogamous species. When it is combined with the breeding system (hermaphrodite, monoecious, dioecious etc.) the difference becomes more apparent (Barrett, 1998; Ogler & Alisz, 2001; Shuster, 2009). For a hermaphrodite self-pollinating species pollen limitation is usually less severe, while for a monoecious species the pollen limitation can be comparatively very high.

Apart from these, there are other ecological factors too which influence the pollen limitation for a species. Floral longevity is also one of the factors responsible for the decrease in pollen limitation, as longer the flower survives the more is the chance for it to be pollinated.

**Bet-hedging strategy** is another factor which induces pollen limitation (Chamberlain, 2007; Delph & Sutherland, 1984; Sutherland, 1986a). The plant puts out a larger number of ovules per flower, as increasing ovule number in a flower is not expensive, to use the occasional “good years” when there is abundant pollination (A. T.-lynn Ashman et al., 2004; Robert Wyatt, 1981).

## **Pollen Limitation meets Resource availability**

We have considered pollen limitation and resource limitation as separate factors but they are not necessarily mutually exclusive of each other.

When the resources are in abundance, pollen becomes the limiting factor for reproduction as all flowers may not get enough pollen to fertilize all the ovules. Conversely, if the resources are limited then no matter how much pollen a flower receives, the plant is unable to initiate fruits from all the fertilized flowers. Hence the fruit set is affected (Casper & Nisenbaum, 1993; Knight et al., 2005; Wesselingh, 2007).

A plant is usually assumed to be a single unit with a collection of flowers and resources. In which all the flowers gets pollinated uniformly and equal resources are available for all the fertilized flowers (Casper & Nisenbaum, 1993; Diggle, 1995; Sutherland, 1986b; Wesselingh, 2007). This assumption can lead to the hypothesis that the whole plant will be either affected by resource or pollen limitation at a time, which is not the case.

An individual plant tries to maintain equilibrium for resource and pollen limitation at the same time to maximize fruiting. As the flowers are not uniformly pollinated across the tree, some gets pollinated before others and the plant relocates all the resources towards it, hence there will be both pollen and resource limitation acting on different parts of the plant at the same time (A. T.-lynn Ashman et al., 2004; Casper & Nisenbaum, 1993; Diggle, 1995; Sutherland, 1986b; Wesselingh, 2007).

## **Phenology**

Phenology is one of the mechanisms by which plants affects the percentage of fruit set. Plants regulate the time and duration of flowering, to maximize the resource availability, pollinator availability, avoidance of pre-dispersal predators etc. (Elzinga et al., 2007; Schaik et al., 1993; Stephenson, 1981; Sutherland, 1986a, 1986b; R. Wyatt, 1982), hence, affecting the fruit set.

The timing of any phenological event is determined by various proximate or ultimate factors. The proximate factors can be the cue for initiation of the event (Abe, 2001; Elzinga et al., 2007; Sabat & Ackerman, 1996; R. Wyatt, 1982). For example, the duration of the day can be a cue for the start of a flowering season or the humidity in the air can be a

cue for monsoon season and fruiting can start. The ultimate factors are the ones because of which the plants can adapt for a particular timing of an event, like optimal resources availability, pollinator availability, match the phenologies of other individuals of same species, avoiding pre-dispersal predator or predator satiation etc. (Abe, 2001; Elzinga et al., 2007; R. Wyatt, 1982).

**A. Optimal Resource availability:** Flowering phenology is matched to the maximum amount of optimal resources available for the growth and reproduction (Stephenson, 1981; Sutherland, 1986b; Wesselingh, 2007).

**B. Pollinator availability:** Flowering time and peak of an individual is selected to match the peak availability of pollinator so that maximum pollination may take place (Delph & Sutherland, 1984; Sutherland, 1986a). The flowering phenology of neighbouring individuals can synchronize to make the group of trees look more attractive and more pollinator visitation may take place (Delph & Sutherland, 1984; Stephenson, 1981; Sutherland, 1986a).

**C. To attract pollinators:** The neighbouring species which have same pollinators or dispersers may synchronise the flowering and fruiting phenologies to attract more pollinators and dispersers towards them cumulatively (Delph & Sutherland, 1984; Schaik et al., 1993; Stephenson, 1981). The trees opt for synchronising when they alone are not able to attract enough pollinators and synchronising with the neighbour can increase the chances of pollination.

**D. Avoiding pre-dispersal Predator and Predator Satiation:** The flowering time can be selected to avoid the peak predator availability. The individuals can flower or fruit very high in number to satiate the predators.

Flowering, fruiting and leafing are three important phenological transitions in plants (Schaik et al., 1993). These three phenophases, though separate, are not mutually exclusive. The occurrence of one influences the other. For example, flowering always precedes fruiting (Schaik et al., 1993).

There is synchrony and asynchrony in flowering time and duration between neighbouring species to either (Schaik et al., 1993). Individuals either synchronise the flowering

to start and peak at the same time or stagger it to be asynchronous with their neighbours. The reasons for that can be:

- A. *Pollinator availability:*** This hypothesis deals with the selection of staggering phenologies between species. Species that share pollinators may have separate flowering season to avoid competition for pollinators and, to reduce the chances of producing low fitness hybrids of two different species (Schaik et al., 1993; R. Wyatt, 1982). This factor may come into picture when the pollinators or dispersers are limited in number and trees compete for them.
- B. *To attract pollinators or dispersers:*** The neighbouring species which have same pollinators or dispersers may synchronise the flowering and fruiting phenologies to attract more pollinators and dispersers towards them cumulatively (Delph & Sutherland, 1984; Schaik et al., 1993; Stephenson, 1981). The trees opt for synchronising when they alone are not able to attract enough pollinators and synchronising with the neighbour can increase the chances of pollination.
- C. *Predator Satiation:*** When the neighbouring species flower or fruit together it also helps in satiation of the predators. The predator can attack both the species for food and they can share the losses due to predation among themselves (Delph & Sutherland, 1984) with low amount of damage to one single species.

### **Architectural effects**

The allocation of flowers and fruits on an individual are not uniform. There is a variation in the number of flowers or fruits position from the apex to the base and also in the number of fruits and flowers across various parts of the tree (Diggle, 1995; R. Wyatt, 1982).

**Integrated Physiological Unit:** Casper and Nisenbaum (1993) introduced the concept of integrated physiological units (IPU's). According to them, the plant should not be considered as a single unit, but a collection of smaller units with their own collection of flowers and leaves. Each IPU functions individually to collect resource and initiate fruits (Casper & Nisenbaum, 1993).

Hence it is safe to say that the amount of pollen any flower receives in and between IPU's can be different. As we have already established that some flowers may be fertilized before others, they become the sink for the resources from the entire plant (Diggle, 1995). So, some IPU's get more resources from the source than others. Hence we can see a difference in fruiting across different IPU's.

### **Temporal variation**

Temporal variation also affects which flower or fruit should receive more amount of resource. As there is a temporal variation in the anthesis of a flower, some fruits are initiated earlier than the rest and the older fruits have higher affinity to attract resource towards itself. So early initiated fruits act as a sink to all the resource and younger fruits have less chances of survival (Diggle, 1995; Stephenson, 1981). This results in a reduced fruit set.

### **Predation**

Predation is also identified as an important factor affecting the fruit set (Elzinga et al., 2007; Jansen, 1971). These are of two types: pre-dispersal predation and post dispersal predation.

#### **Pre-dispersal Predation**

Pre-dispersal seed predation occurs before dispersal. Even after the fruit has been dropped by the plant, if it is predated before getting manipulated by the disperser, it can still be termed as pre-dispersal predation (Jansen, 1971).

Pre-dispersal predators are mostly Small sedentary specialist feeders (Crawley, book). Pre-dispersal seed predators utilize specific cues like plant chemistry (volatile compounds), flower/ fruit colour, and size to identify their hosts (Jansen, 1971).

In addition to the plant specific cues, these predators also tune their phenology to match seed production (Parachnowitsch & Christina M. Caruso, 2008). Therefore, animals with shorter life span, like insects, are more common pre-dispersal predators. Some common types of pre-dispersal predators are Diptera, Coleoptera, Hemiptera, Hymen-



optera and Lepidoptera (Crawley, 1989). Apart from these, there are important vertebrate pre-dispersal predators, especially birds and small mammals.

There is wide variation in floral traits in plants. Pollinator mediated selection alone doesn't explain the variation in flower characteristics. Pollinators and predators both shape the evolution of floral traits and plant design (Cariveau et al., 2004).

As pre-dispersal predators also use the same cues as pollinators, both these factors pose an opposing effect on the plants. In some cases it is observed that predators exert greater selective pressure than pollinators (Brody, 1997; Cariveau et al., 2004). It has been observed in some studies that there is weak effect of floral trait to pollinator visitation and pollinator visitation to seed set (Cariveau et al., 2004).

Phenology has been observed to be the main cue of both pollinators and predators. High predation during peak flowering season, results in a shift of peak flowering towards early or late flowering (Elzinga et al., 2007).

If the predation is constant throughout the season, the best bet for the species is to produce a large amount of flowers synchronously in order to satiate the predator (Elzinga et al., 2007; Jansen, 1971)

Another common response to predation is re-absorption and abortion of fruit. It is compensated by greater growth of the remaining fruits (Jansen, 1971).

Seed Predation has been observed to be a major selective force which affects seed morphology, seed chemistry, flowering, fruiting and dispersal behaviour (Andersen, 1989).

Pre dispersal predation can also affect the abundance and distribution of the plant species. They can affect seed number directly by feeding but also by affecting the density of safe sites available for the seeds to grow. Although it has been observed that pre dispersal predators affect relative reproductive success of the individual, it does not make a great difference to population size (Andersen, 1989; Ehrlén, 1996).

Pre-dispersal predation varies from individual to individual, site to site and year to year. There is a clear spatio-temporal variation in the extent of damage to the plants (Andersen, 1989; Ehrlén, 1996). Although, some studies (Zimmerman, 1980) have shown consistencies to damage across years.

### **Post-dispersal predation**

Post dispersal predation occurs after the seeds have been dispersed (Jansen, 1971)

It has been reported that on average survivorship is around 55% for pre-dispersal and 50% for post-dispersal predation of seeds (A. T. Moles et al., 2003).

As the dispersal patterns are not similar, the pattern in post dispersal predation varies. This variation can be observed with respect to distance from the parent tree (Hulme, 1994; Jansen, 1971; Schupp, 1988), or site (Crawley, 1989; Feer & Forget, 2002; Hulme, 1994; Schupp, 1988), species (Crawley, 1989), seed size (Crawley, 1989; A. T. Moles et al., 2003), burial depth (Crawley, 1989; A. T. Moles et al., 2003; Schupp, 1988), seed density (Hulme, 1994; Jansen, 1971; Schupp, 1988) and season (Hulme, 1994). Predation also varies across years (Crawley, 1989; Feer & Forget, 2002; Hulme, 1994; Schupp, 1988).

As the seed size increases the chances of predation increases (Hulme, 1994; A. T. Moles et al., 2003). Large seeds spend more time on soil surface than smaller seeds also that it's better for a predator to forage on few large seeds than a large amount of small seeds (A. T. Moles et al., 2003). Also, if the seed is buried deep inside the soil, it becomes hard for the predators to dig up and eat them (Crawley, 1989; Hulme, 1994; A. T. Moles et al., 2003). But the seed size also makes an impact on the seed burial, if the seed is larger, then it stays on the surface longer than smaller seed hence chances of predation increases (Hulme, 1994; Schupp, 1988). The vulnerability to predation may also depend on the nearness to a log or trunk, the size of the nearest conspecific adult or whether a seed is in a treefall gap or the understory and the seed density (Schupp, 1988).

Seed dispersal and germination are also part of the reproductive process. But my study deals only with the pre-dispersal part of the process.

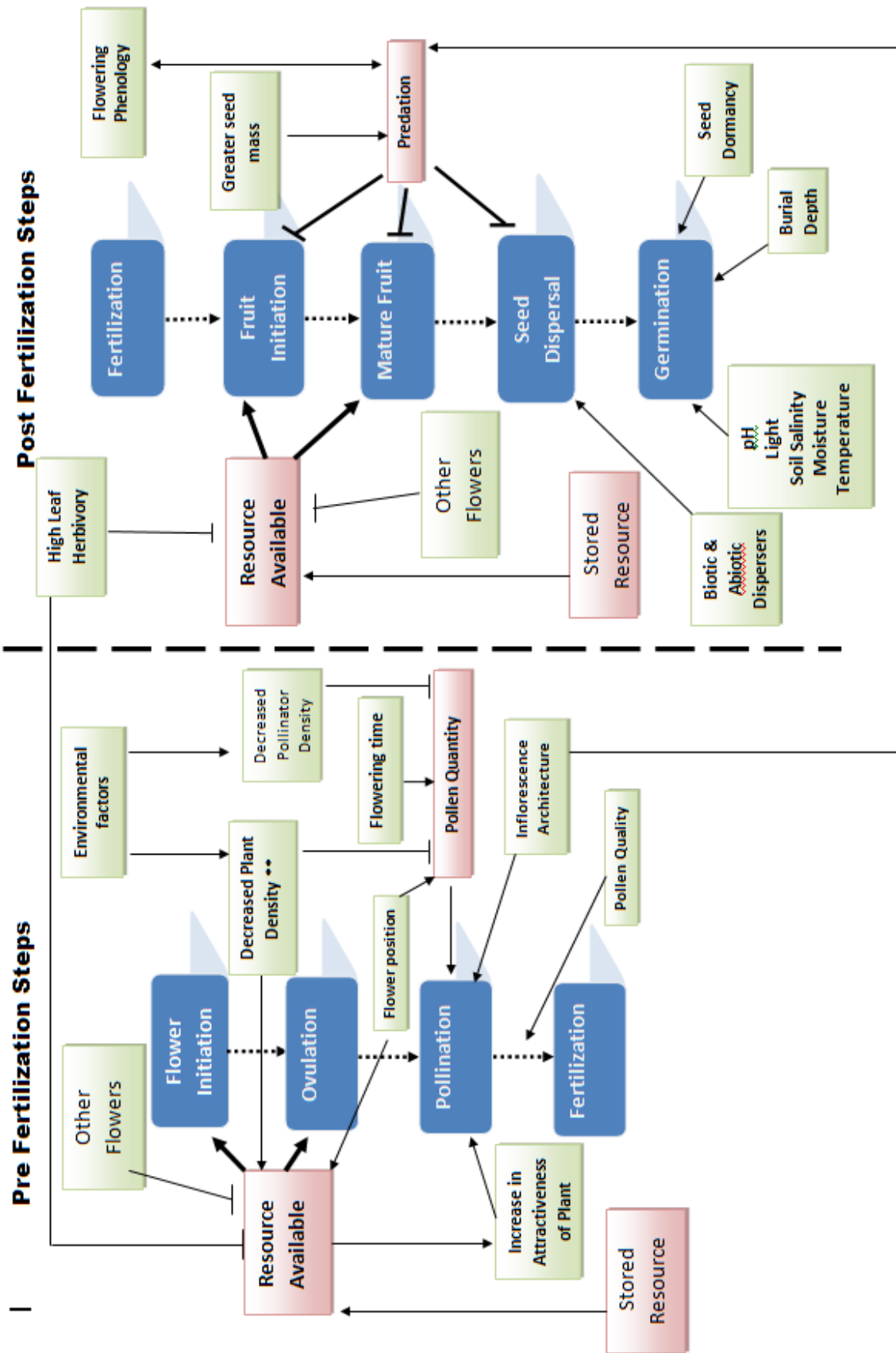


Fig 1: All the factors affecting the reproduction at various steps

## AIM OF THE STUDY

### Study site and species

The species, *Randia dumetorum* was observed in areas surrounding Bhimashankar wild life sanctuary (19.0821° - 19.0853° N, 73.5518° - 73.5559° E), Maharashtra State, situated in Northern-Western Ghats of India.

The Western Ghats cover about 180,000 km<sup>2</sup>, extending from Gujarat to Tamil Nadu. Mean annual rainfall ranges from 900 mm to 5000 mm or more.

Western Ghats showcases four different forest types: moist evergreen forests, semi evergreen forests, moist deciduous forests and dry deciduous forests. The vegetative cover in Bhimashankar is mainly moist deciduous forest, although some regions show a mix of wet evergreen forests and dry deciduous forests. The site under the consideration of my study is the moist deciduous forests.

Bhimashankar constitutes the northern boundary of the Western Ghats. Our study site was at elevation between 958 m to 1045 m. Because it is a highland area located below the subalpine zone the mean annual rainfall received is between 2000 mm to 3500 mm.

Within our study site there are three different locations varying in the number of trees on each site and elevation. The three sites include *Husa* (elevation: 958 – 978 m), *Husa-Hill* (elevation: 983 – 990 m) and *Sheel* (elevation: 1020 – 1045 m). Husa is a plateau region having varying terrain. Most of the site is visibly rocky and very low number of the study species is present in isolation. The boundary of the site and some parts in the middle has deeper soil, so more resources are present hence these locations has higher plant density. Husa-Hill site is mostly dense forests as the soil depth is higher. The trees are present mostly on the edge of the forests or inside it. Sheel is the highest of the three locations and the rockiest of all. The trees are dispersed at a larger area with patches of vegetation.

### About study species:

The plant studied was *Randia dumetorum* (Common name- Ghela, Mainphal): Family - Rubiaceae. It is found all around India up to 4000 ft. Altitude, from Himalayas in Kashmir to east wards. It is also seen in Gujarat, Tamil Nadu, *Suralik* Range, Maharashtra,

Bengal, Bihar and Orissa. It is mostly found in wet and moist deciduous forests of India. The study was conducted from March, 2010 to Feb, 2011.

It is a large deciduous thorny tree which extends up to 10 meters in height. The leaves are wrinkled, shiny and thick when matured. The flowers are small, fragrant, solitary (usually in a bunch of 2-3 max), and white in colour (turns yellow as the flower ages). The flowers are hermaphroditic in nature i.e. contains both male and female reproductive parts. The fruits are smooth, globular and round with longitudinal ribs, the colour is yellow when ripe, and fruit size is usually ranging from 0.5 cm to 2.5 cm in diameter. The seeds are large in number (ranging from 60 to 150) embedded in the dark pulp.

**Other information of *Randia dumetorum*:**

The flowering season for the species extends from mid March up to late June in Bhimashankar (before the monsoon season starts). Sometimes flowering has been observed after the end of flowering season also, but that is considered to be an exception. During the flowering season at our sites about 69% (282 trees out of 409) of the trees have been observed flowering.

Fruiting is observed on the tree for almost whole of the year. New fruits can be seen on the tree as early as mid of May. Maximum number of fruits on a tree ranges from 1 to greater than 200. The fruit dispersal starts from the end of December and it extends up to end of May.

Some of the dried fruits had visible pre-dispersal predation marks like exit holes and web like structure to artificially hold the flower to the branch. One of the predators has been identified as a butterfly *Virachola perse ghela* (Tamil Large Guava Blue). There are also signs of another unidentified predator.

**About Pre-Dispersal predator:**

There are some pre-dispersal predators observed infesting the fruits of *Randia*. One of them is identified as *Virachola perse*.

*V. perse* is found from the Himalayas to the south. These are found plentiful near the sea coast of Kanara and extend up to the east till the jungles last. The most common host plant is *Randia dumetorum* (Bell, 1927).

They lay their eggs in the flowers of Ghela (*R. dumetorum*). Then the larvae grows inside the fruit as it matures, it feeds on the growing seeds. The larva makes a small hole when the fruit is immature to escape later as the stony hard surface of the mature fruit is tough to pierce. From this hole, the larva periodically comes out of the fruit to weave silk thread around the fruit to fasten it with the stalk. It is done to safeguard the fruit from falling off, when the fruit is aborted. It is dangerous for the larva if the fruit is dropped on the ground before it matures, as the fruit rots early, ants start coming in the fruit hence making the fruit uninhabitable for the larva. When the butterfly matures it widens the hole and escape from the fruit.

We have observed only one larva in one fruit, and have also noticed ants going in and out of the fruit, purpose unknown. It can be assumed that the ants were scavengers not visitors. The fermented sugar of the fruit can be one of the attractants of the ants.

Some investigations also state that the ants serve as the attendant of the larvae. The ants enter from the hole bored by the larvae and the excrement of the larva, which would otherwise have filled up the hole, was presumably removed by the ants in order to allow themselves entrance. Of course, it is quite possible that the larva itself removed the stoppage by backing, as it must have done where no attendant ants were found.

We tried to understand the various factors which affect the fruit set in *Randia* and try to find out the specific factors affecting fruit set in *Randia*.

Studies have shown the importance of resource availability (current and stored) in determining the fruit set of a plant. We used the site and height of the tree as a proxy for the resource availability. Assuming, more the resources present more is the height of the tree. And the higher the tree the more will be the resources accumulated by the tree as roots can go deeper and it can put out more number of leaves.

We used fruiting information from 2010 to examine the amount of stored resource in a tree. The working assumption being if the number of fruits is higher in a year then the amount of resources stored must be lower and the fruiting in the next year should be lower.

It is stated in earlier studies that there is a relationship in fruit size and number depending on the resource availability, cost of fruits etc. So, we also studied the relationship between fruit number and fruit size.

We also examined if the resource allocation is uniform in the tree or branch-specific. This can be identified by checking if the fruiting is uniform or not across the canopy. We studied this to identify if *Randia* is also divided into different IPU's as we observed that in many trees the fruiting was not uniform.

We examined the relationships between flowering phenology and flower or fruit number. Phenology is determined by factors like resource availability, pollinators, dispersers, predators etc. So, if phenology affects the flowering or fruiting, we may get a better understanding of the other factors that affect fruit number.

As predators are also one of the factors which affect fruit set directly by damaging the fruits. We examined the proportion of infestation per tree and its relationship to factors like flowering phenology, flower number and fruit number.

## **MATERIALS AND METHODS**

### **Data collection methodology**

On the study site, there are 481 trees marked out of more than a thousand trees observed. The data was collected for information about flowering, fruiting, leafing, predation and surrounding habitat.

### **Flower information**

The flowering data has been collected in Qualitative (Yes/No, lot/few, colour and drying) and Quantitative (flower number and bud number) form. The data has been accumulated from phenology observations; transect data; whole site censuses and data recorded for different experiments. This data has been collected in the flowering season of 2010 (Somanathan, H. et. al., unpublished)

This information has been used to estimate flowering phenology, duration of flowering per tree, cumulative flowering number for the season, peak flowering date and number. Methodology of which is described later.

### **Fruit information**

The fruit censuses were carried out in a monthly fashion. The censuses were taken on the end of each month from May, 2011 to February, 2012. The data included the fruit count, maximum fruit size, dried fruits (if any), and fruits on ground (if any).

The fruit number and dried fruits recorded were actual count of the fruits present on the tree at the time of the census. On certain trees when the number of fruits were very high (>60) a rough estimate has been taken by taking the fruit count for half of the canopy and then doubling the result. It gives a rough estimate for the fruit number. In this case the dried fruits were also taken as a percentage of the total fruits counted.

For fruit size, the largest fruit size we can observe has been recorded. For simplification purposes a resolution of 0.25 inch has been used.

The area under the canopy of the tree had been scanned for any fruits present on the ground. It helps us to check if the fruits on the ground are aborted due to infestation or they are dispersed fruits.



### **Seed information**

To get information on seeds the fruits were collected from the tree on 10-May-2011 and from the ground on 3- Feb-2012 census. The fruit size was recorded from those. Then the fruits were cracked open to collect the seeds and counted to get the seed number in each fruit.

### **Neighbourhood information**

The surroundings of each tree was studied to get the information of neighbouring species, number of neighbours and the number of sides of canopy of the tree shaded.

For the shade information, the canopy top was considered as a square and the directions where it was covered by neighbours was recorded as North, South, East or West. We can use the information of number of sides shaded and direction of shade to find out patterns.

Tree shade can be quantified as follows:

- a. Open : If the tree is not surrounded by any neighbours
- b. One side: If the tree is surrounded by one neighbour from one side
- c. Two sides: If the tree has two sides covered
- d. Three sides:
- e. All side covered:

## Estimating Phenology

### Method to estimate flowering phenology

To estimate the flowering phenology, all the quantitative and qualitative information about the presence and number of flowers were collected and composed in one file date-wise. The qualitative information was of the form 'Yes/No' (1/0) and the quantitative information is the number of flowers. Then the following rules were used to identify the flowering phenology of a tree:

1. If Buds but no flowers present on X (date) – Then flowering date was estimated as X+7(date)
2. If the fruiting has happened, then we assume that flowering has happened regardless of non availability of flowering data.
3. Flowering start:
  - a. If the flowering goes from no to yes (0, 0, 0, 1....)
    - i. If sampling interval 14 days or less – Flowering Onset
    - ii. If sampling interval greater than 14 days
      - a) If the flower number at yes is >10 then no information
      - b) If the maximum flowering is ( $\leq 50$ ) and at yes flowering the number is <10% of maximum, it is the flowering onset.
      - c) If the maximum flowering is ( $\leq 50$ ) and at yes flowering the number is >10% of maximum, it is not the flowering onset.
  - b. If it is no data and flowering yes (-, -, -, 1):
    - i. If the yes flowering is on 25<sup>th</sup> Mar 2011 or 30<sup>th</sup> Mar 2011:
      - a) If the number of flowers is low ( $\leq 10$ ) then flowering onset is 25 Mar 2011 or 30 Mar 2011
      - b) If the number of flowers is high (>10) then flowering onset will be 1 week prior.
      - c) If information is only (yes/No) and no information on number of flowers then consider flowering onset.
    - ii. If the yes flowering is on some other date
      - a) If the flower number at yes is >10 then no information

- b) If the maximum flowering is ( $\leq 50$ ) and at yes flowering the number is  $<10\%$  of maximum, it is the flowering onset.
    - c) If the maximum flowering is ( $\leq 50$ ) and at yes flowering the number is  $>10\%$  of maximum, it is not the flowering onset.
  - c. If it is no flowering then missing data then yes flowering (0, -, -, 1)
    - i. If interval between 0 – 1 is  $>14$  days then no information
    - ii. If interval between 0 – 1 is  $\leq 14$  days
      - a) GOTO Rule no (4-b-ii)
- 4. Flowering end:
  - a. If the flowering goes from yes to no (1, 1, 1, 0....)
    - i. If sampling interval 14 days or less – Flowering End
    - ii. If sampling interval greater than 14 days
      - a) If the flower no is 20 or less and the colour of flowers is yellow then flowering ends 7 days after that
  - b. If it is flowering yes then no data (1, -, -, -)
    - i. If it is last flowering on 8-may-2011 or 13-may-2011 (Yes/No information) and then no data available
      - a) If on the 1-May census the flower number is  $\leq 50$  and yellow in colour and no buds then the flowering ends on 8 May
      - b) If flowering on 1-May-2011, all yellow flowers, buds present and no flowering on 13-May-2011. Then flowering end date will be 13-May-2011
    - ii. If last flowering on some other day
      - a) If the flower number is 20 or less and the colour of flowers is yellow then flowering ends 7 days after that
  - c. If it is yes flowering then no data then no flowering (1, -, 0)
    - i. If interval between 0 – 1 is  $>14$  days then no information
    - ii. If interval between 0 – 1 is  $\leq 14$  days
      - a) GOTO Rule no (5-b-ii)

If there are conflicts in data from two different sources

1. If the conflict is due to two different data on the same date and one of it is a projection from the buds. Then use the actual census data instead of the projection
2. If there is missing data between two census dates and both have same value then the missing information will be same as that of its neighbours. The sampling interval should be less than 14 days otherwise no changes. For E.g. (1, -, 1) = (1, 1, 1)
3. If the data is of the form (0, 1, 1, 0, 0, 0, 1, 1, 0) or any other similar data in which there are more than two onset and end, just take the first onset and the last end and ignore the rest.

For Peak flowering

1. Minimum 3 data points should be available for a tree
2. The difference between any two flowering data points should not be more than 14 days.

To estimate cumulative flowers:

1. Selection of trees: Trees were selected which had a start date and an end date. Also, there should be at least three data points in the calculation and the duration between any two points should not be more than 14 days.
2. Calculation of total flowers: The graph of flower number and time (days) was drawn, then the area under the curve was calculated. That area under the curve was divided by the life span of flowers which is eight days (Hema Somanathan, personal communication).

### **Fruiting phenology methodology**

To estimate the fruiting phenology the fruiting start date and fruiting end date has been noted down from the fruit number data. It is a very rough estimate of the month in which the fruit initiation started. This data provides us with the pattern of fruiting observed. We can use fruiting phenology to find the relation of fruiting with other factors like:

- a. Flowering duration- if the flowering duration affects the fruiting time;
- b. Fruit number- if the no of fruits present affects the fruiting duration and abortion rate.

- c. Abortion rate- if the timing of fruiting affects the abortion rate

The following rules have been used to estimate the fruiting phenology:

1. The fruiting censuses are written in the order of the month.
2. If for any tree a definite start and stop is observed only that tree has a start or stop information. Ex. (0, 0, 1,) start ; (1, 1, 0) stop
3. If for any tree there is a missing information and a start (-, -, 1)
  - a. Then the 1 can be a start if the fruit number is either <5 or <25% of the maximum, whichever is lesser.
4. If for any tree there is a missing information and a start (1, -, -) then there is no information on stop
5. For the trees which haven't been census before February will have no information on flower start but they might have a stop
6. For fruiting duration, the tree must have a fruiting start and a stop

### **Method to estimate Branch-specific fruiting**

The uniform fruiting expects that the fruit will be evenly distributed among various parts of the tree, while branch-specific expects a variation.

- For this experiment, I will be selecting different groups of trees on the basis of fruit number. That is low, medium and high fruiting.

The groups of trees are made due to the fact that the experimental data will be affected by the no of fruits on the trees. Trees with low fruit # might give different result than the trees with higher no. So to cover the whole spectrum the different groups and range of fruit numbers might help. Also, there might be some variation in the result due to high or low no of fruits.
- These groups will be based on the no of fruits
  - o Group 0- Very Low fruiting – Fruit number from 3-5
  - o Group 1- Low fruiting- Fruit number between 5-10
  - o Group 2- Medium Fruiting - Fruit # between 10- 20
  - o Group 3- High Fruiting - Fruit # between 21 to 35

- More than 35 it will be harder to track
- The data from low fruiting trees (say 1 or 2 or 3) was rejected to be used in this experiment as the result of being branch-specific can be just an artefact.
- Height of the tree will also be taken into account for data selection, i.e. very high trees (Height > 5.5 m) will not be selected due to practical issues in counting and tracking the branches.
- No of trees to be used from each group =
 

Group 1	3 to 5	25
Group 2	6 to 10	14
Group 3	11 to 20	11
Group 4	21 to 38	11

To estimate whether it is branch-specific or uniform, we used the following rules:

1. The various fruiting and non-fruiting branches of the tree were identified
2. Each branch was given a numerical value. This value is equal to the fraction of the whole canopy it covers.
3. Each fraction of the canopy was identified and the number of fruits (green and dry), on the branch were counted.

To be a uniform fruiting tree the ideal fruit number per canopy units must be same in a tree. If there is a variation in the distribution of fruits on the tree then we can say its branch-specific. We used Chi-square test to identify the same.

## **Statistical Methods used**

The data was log transformed when it was not normally distributed. Microsoft Excel 2007 was used to do all the statistical analysis.

The statistical method used for testing the correlation between two factors was Pearson's correlation coefficient test. This is a parametric test for normally distributed data. The P-value of significance we used as a reference is 0.05.

To find the variation of a data according to a single factor, ANOVA was applied. The reference P-value of significance was 0.05.

For the qualitative comparison of different years' fruiting and branch-specific fruiting experiment, we had to look at the variation of the observed data from the expected. Thus, a chi-square test was applied. A p-value of 0.05 was taken to be the cut-off for significance.

## RESULTS

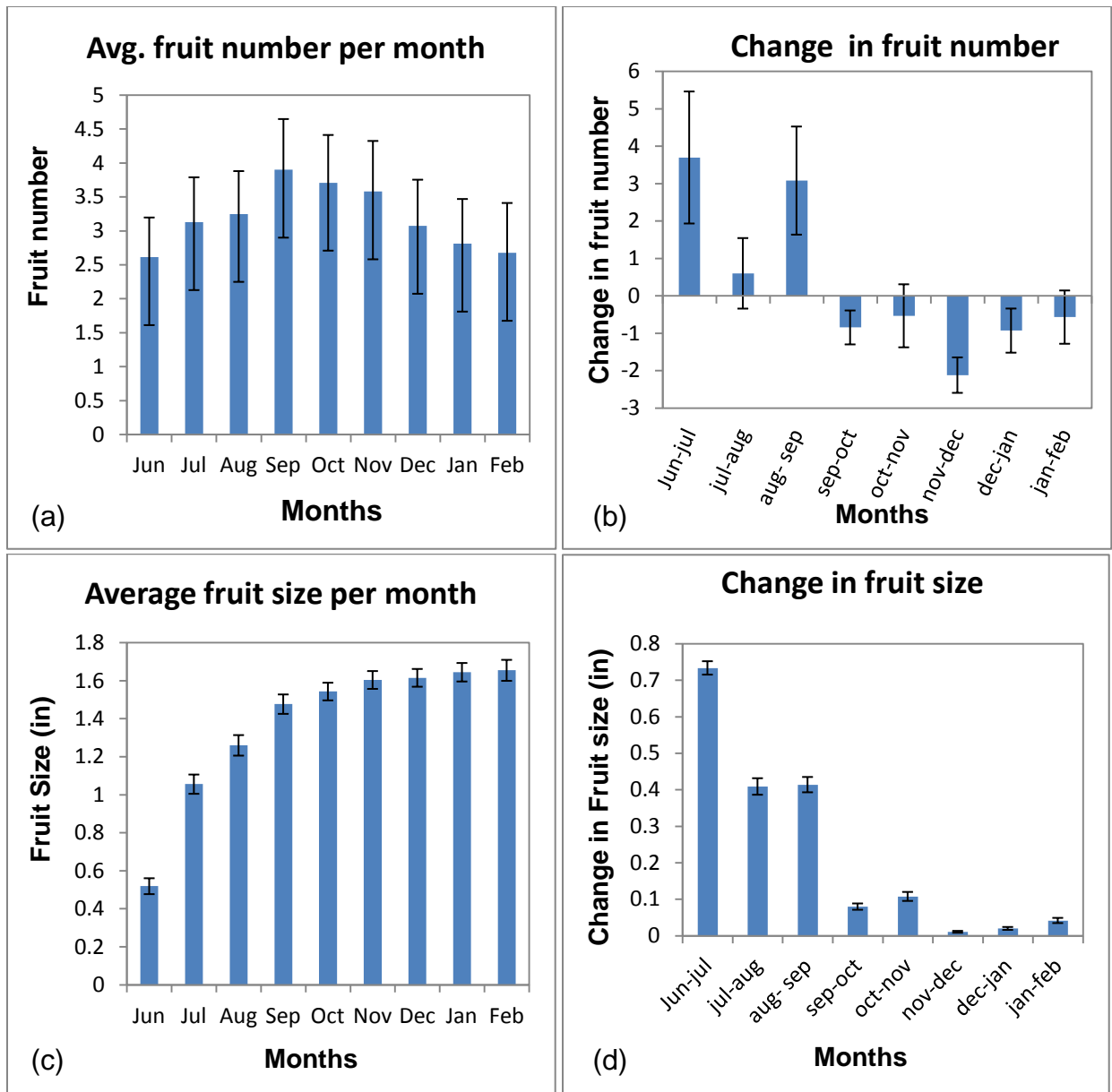
### **Growth pattern through the season**

Across all three sites average fruit number (Fig. 2(a)) shows a gradual increase till September and then it started decreasing. The average change in fruit number (Fig. 2(b)) was positive in the start of season till September and then it became negative, i.e. the fruit number was increasing till September and then it started decreasing.

We can observe that there is a sharp increase in fruit number in July and September and a sharp decline in December.

Average fruit size (Fig. 2(c)) shows an asymptotic curve increasing sharply in the start and then gradually saturating to a peak and the average change in fruit size (Fig. 2(d)) was positive all throughout the season, but the rate of change decreased. Also, we can see a sharp increase in the fruit size in July then the growth of the fruit started declining and reached at a constant maximum level by November.





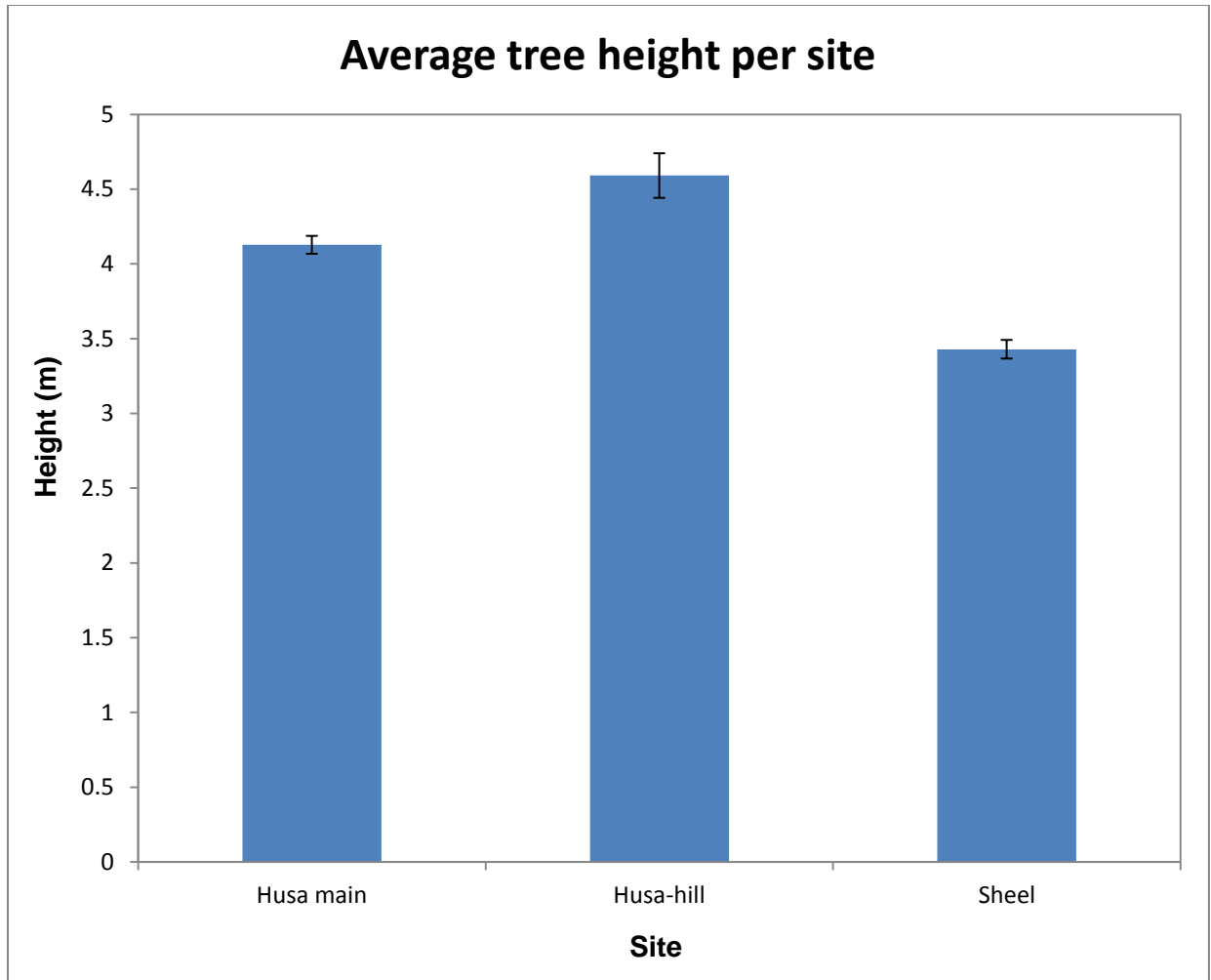
**Figure 2:** Growth pattern for fruit number and fruit size through the entire season (June, 2011 to Feb, 2012) :- (a) Average fruit number, (b) Average change in fruit number, (c) Average fruit size, and (d) Average change in fruit size per individual was calculated and observed in each month for the entire fruiting season from June, 2011 to Feb, 2012. Error bars represents  $\pm 1$  S.E.

### **Site-wise variation in height and fruit number**

The difference in average height between the three sites was significant (Fig. 3). The average height of the trees was greatest in *Husa-Hill* (4.5 m.), then *Husa-Main* (4.12 m). The lowest height of trees was in *Sheel* (3.4 m).

The proportion of fruiting trees in the three sites was also calculated. The results showed that in *Husa-Main* a larger percentage of fruiting trees are present (40.28 %) and *Husa-Hill* was a close second (32%). *Sheel* had a very low number of fruiting trees (12.15%)

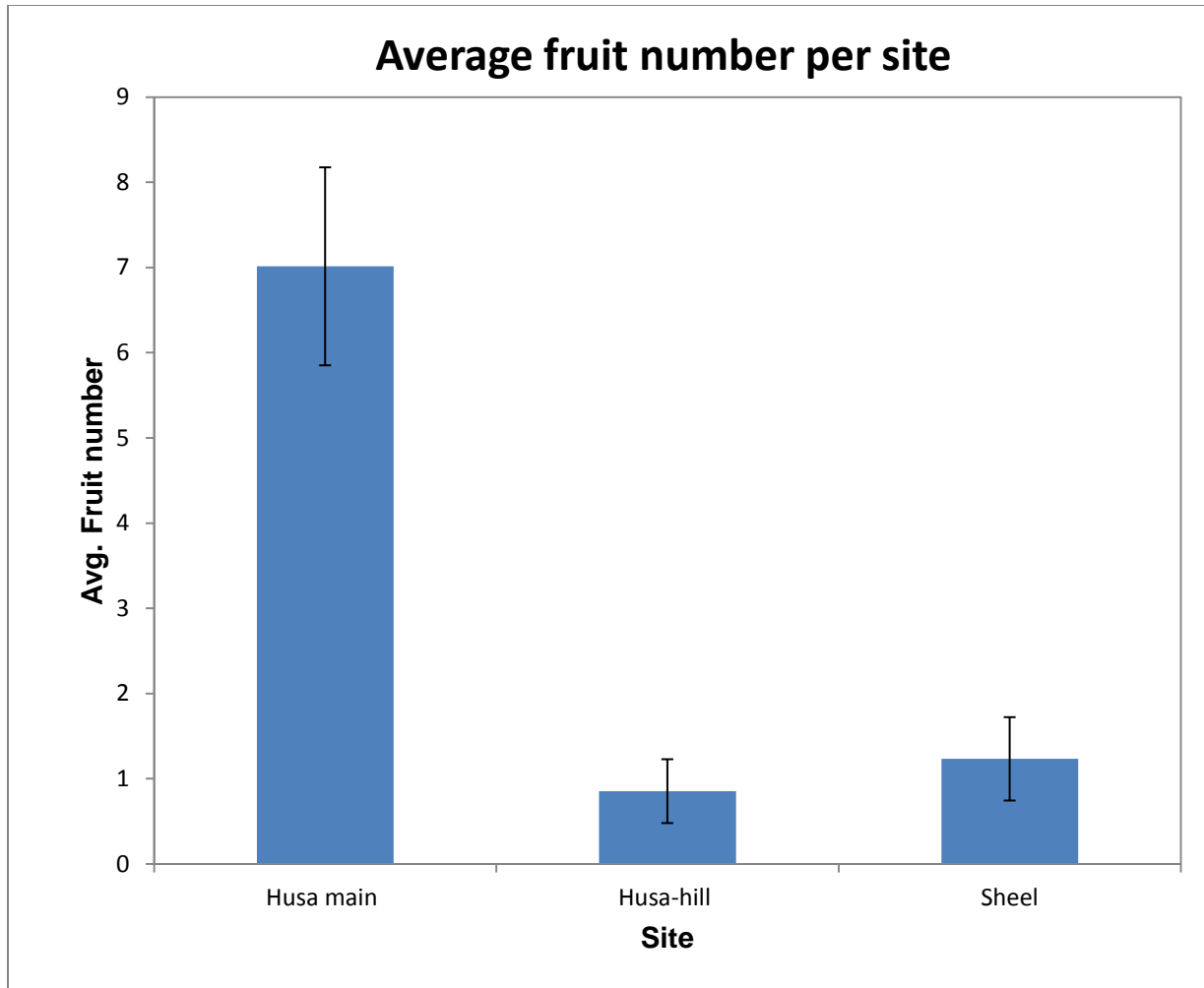
*Husa-Main* has higher number of fruits than the other two sites (Fig. 4). Apart from this, the difference in average fruits with site was also calculated for all fruiting trees to check if the variation of average number of fruits in the three sites varies for fruiting trees. The difference in fruit number for the fruiting trees with sites was not significant ( $F= 1.703$ ;  $P\text{-value}= 0.18$ ).



**Figure 3:** Average tree height with respect to site. Average tree height in each site was calculated and the difference in height between them was analysed using ANOVA. Error bars represents  $\pm 1$  S.E.

**Table 1:** ANOVA table for Site versus Tree height

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3664.686	2	1832.343	6.862711	0.001158	3.015499
Within Groups	121751.9	456	266.9999			
Total	125416.6	458				



**Figure 4:** Average fruit number with respect to site. Mean fruit number per tree in each site was calculated and the difference between them was analysed using ANOVA. Error bars represents  $\pm 1$  S.E.

**Table 2:** ANOVA table for Average fruit number compared site-wise

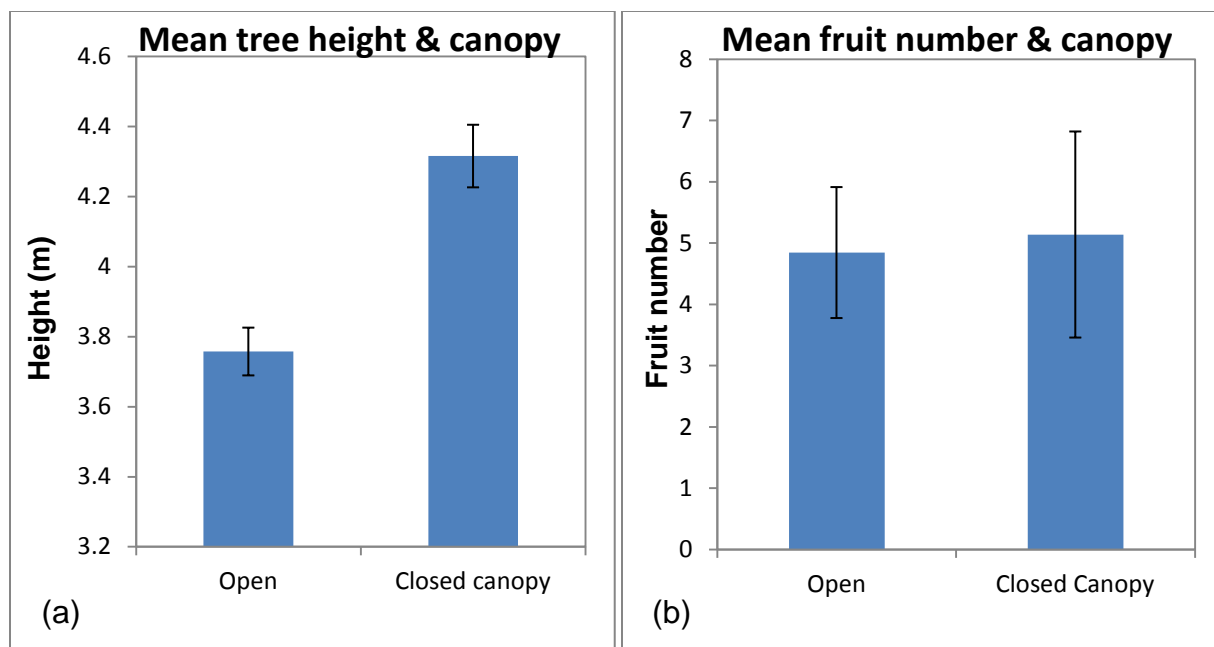
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	58.32	2	29.16	26.6953	1.10037E-11	3.01568
Within Groups	493.732	452	1.09233			
Total	552.052	454				

### **Effect of tree location – Open versus closed canopy**

The height of trees was compared between trees in open or closed canopy conditions. The trees 0, 1, 2 sides of neighbours were taken to be trees with open surroundings. The closed canopy trees are the ones which lie at the edge of the forest or in the middle i.e. 3, 4 sides covered by neighbours.

The difference in height was significant between open and closed canopy (Fig. 5 (a)). Closed canopy trees were higher than trees growing out in open.

The average fruit number was also compared with trees in open and in closed canopy. We found the difference in fruit number was not significant (Fig. 5 (b)). The average fruit numbers are similar for both open and closed canopy trees.



**Figure 5:** Effect of open (isolated) versus closed canopy (within or edge of continuous forest) on: (a) Average tree height and (b) Average fruit number per tree was calculated for all the trees and observed for open and closed canopy. Error bars represents  $\pm 1$  S.E.

Table 3: (a) ANOVA table for Mean tree height and tree location

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	29.56605	1	29.56605	25.51221	6.78E-07	3.865537
Within Groups	449.6525	388	1.158898			
Total	479.2186	389				

Table 3: (b) ANOVA table for Mean fruit number and tree location

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	8.253799	1	8.253799	0.02393	0.877144	3.865474
Within Groups	134173.4	389	344.9187			
Total	134181.6	390				

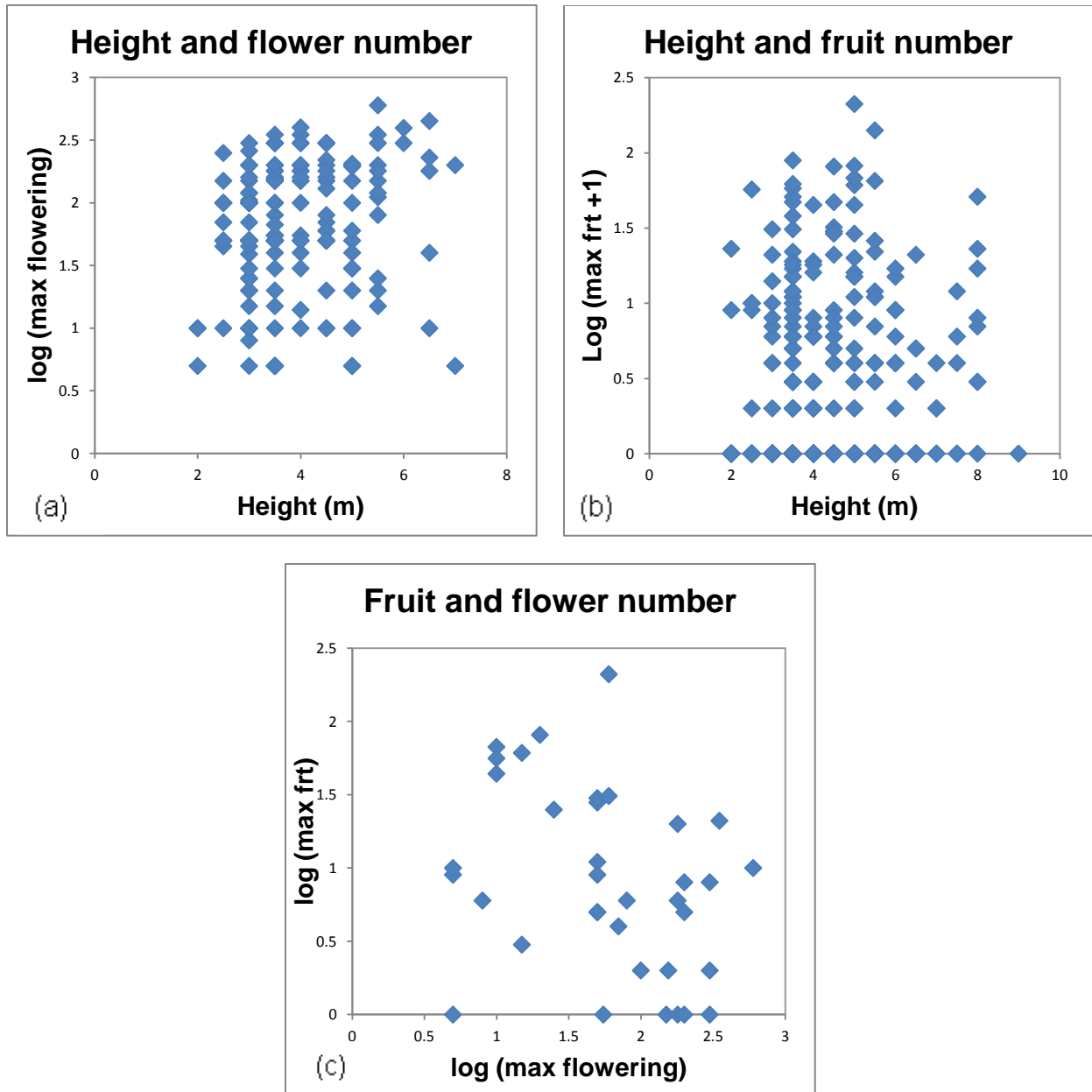
### **Relation between tree height and fruit or flower number**

There is variation in tree height. So the relation between fruit and flower number with height of tree was estimated.

Flower number and tree height were positively correlated (Fig. 6 (a):  $N= 171$ ,  $R= 0.156$ ,  $P\text{-value}= 0.043$ ).

Tree height and fruit number were positively correlated with a significant relationship (Fig. 6 (b):  $N= 475$ ,  $R= 0.153$ ,  $P= <0.001$ ). This data was also examined for raw fruit data ( $N= 475$ ,  $R= 0.09$ ,  $P=0.048$ ) and for fruiting trees (Raw:  $N= 128$ ,  $R= 0.01$ ,  $P=0.906$ ; Log transform:  $N= 128$ ,  $R= -0.043$ ,  $P =0.607$ ).

Fruit and flower number are negatively correlated (Fig. 6 (c):  $N=36$ ,  $R= -0.330$ ,  $P= 0.049$ ). So, if there is high number of flowers the number of fruits will be low for that plant.



**Figure 6:** Relationship between height, flower number and fruit number. Tree heights were compared with (a) flower and (b) fruit number. For fruit number, non-fruiting trees were also used in the analysis. (c) Relationship between fruit and flower number



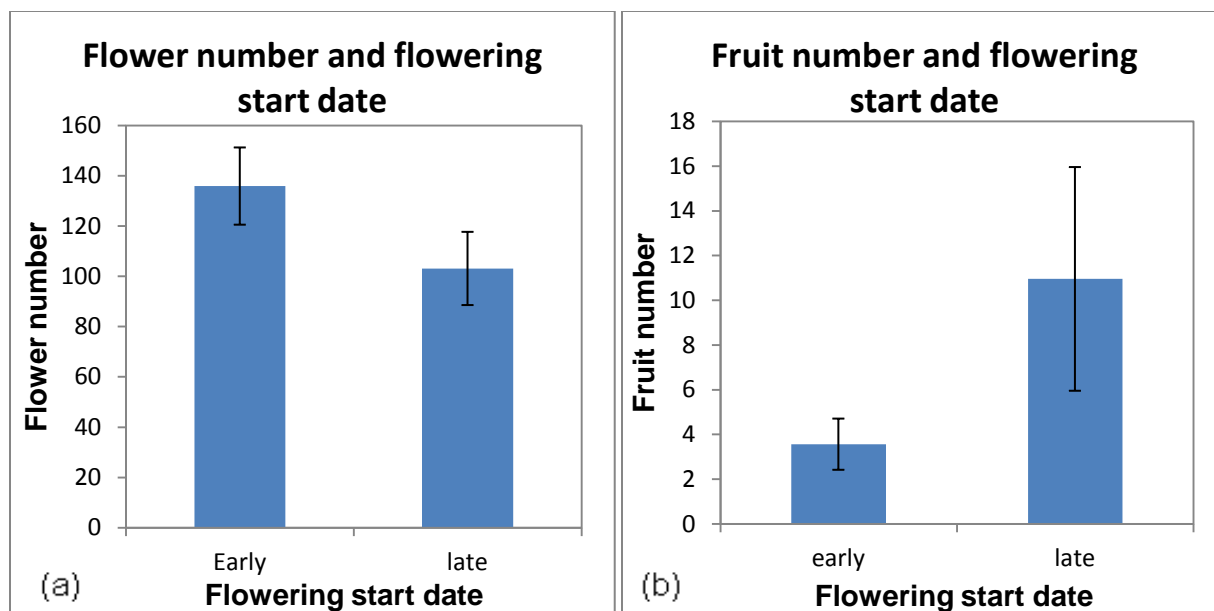
### **Effect of flowering phenology on flower number and fruit number**

The effect of flowering phenology for early (25-Mar to 14-Apr-2011) and late flowering (15- Apr to 8-May-2011) and flowering duration: medium (14 – 28 days) and long (28 days and more) on flower number and fruit number was observed.

The difference in flower number with different flowering start dates and duration was not significant (Fig. 7 (a); Fig. 8 (a)).

The difference in fruit number for early and late flowering was marginally significant (Fig. 7 (b)). Late flowering trees had higher number of fruits.

Also, the difference in fruit number with medium and long flowering duration was marginally significant (Fig. 8 (b)) and trees with shorter flowering duration had higher number of fruits.



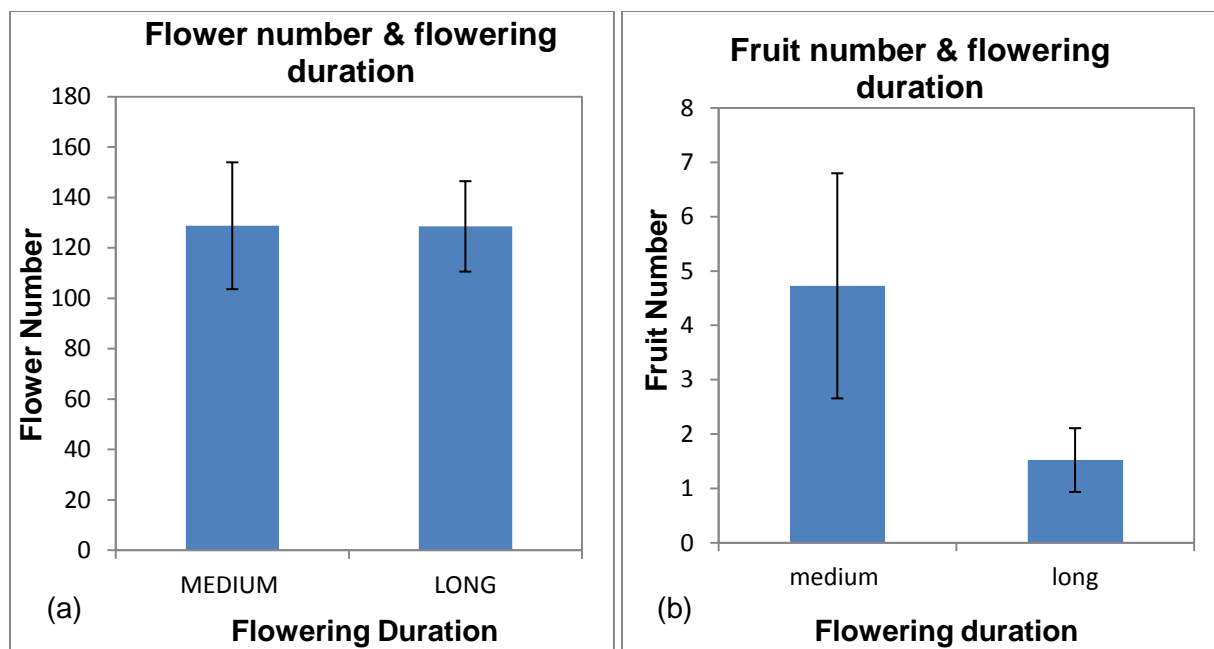
**Figure 7:** Relationships of flowering start date with: (a) Flower number, (b) Fruit number. The difference in (a) Flower and (b) Fruit number with different flowering start date was compared using ANOVA. The two flowering groups were early (25/03/11–14/04/11) and late flowering (14/04/11 and later). Error bars represents  $\pm 1$  S.E.

Table 4(a): ANOVA table for maximum flowering and Flowering start date

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	29402.88	1	29402.88	2.303949	0.131941	3.928195
Within Groups	1391053	109	12761.95			
Total	1420455	110				

Table 4(b): ANOVA table for maximum fruiting and flowering start date

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1566.456	1	1566.456	2.794108	0.097307	3.922879
Within Groups	65032.87	116	560.6282			
Total	66599.33	117				



**Figure 8:** Relationships of flowering duration with: (a) Flower number, (b) fruit number. The two groups of flowering duration are Medium (14-28 days) and long (greater than 28 days). Error bars represents  $\pm 1$  S.E.

Table 5(a): ANOVA table for max flowering and flowering duration

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.8838	1	0.8838	6.5E-05	0.994	3.9959
Within Groups	846569	62	13654			
Total	846569	63				

Table 5(b): ANOVA table for max fruiting and flowering duration

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	150.6136	1	150.6136	3.663255	0.060099	3.990924
Within Groups	2631.341	64	41.1147			
Total	2781.955	65				

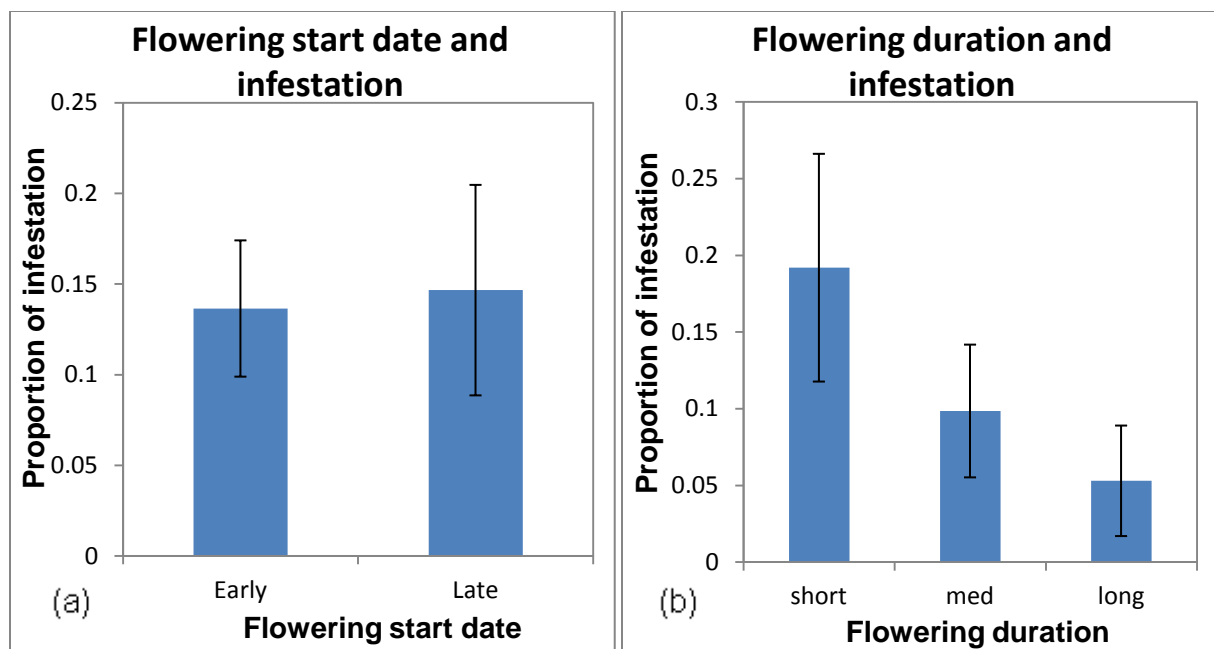
### **Infested fruits and flowering phenology**

The pre-dispersal predators of *Randia* (specifically *V. perse ghela*) select the host at the flowering stage (Bell, 1927), so the relationship between infestation and flowering phenology was checked.

The relationship between flowering start date and infestation was not significant (Fig. 9(a)).

Flowering end date was also compared with proportion of infestation and it was a negative correlation (N=28, R= - 0.410, P=0.03). It means if the flowering ends late the proportion of infested fruits are low.

Flowering duration also had a significant negative relationship with infestation (Fig. 9 (b)). The longer a tree flowers the less is the proportion of fruits infested.



**Figure 9:** Difference in average number of infested fruits for: **(a)** Different flowering start date. The two flowering groups are early (25/03/11– 14/04/11) and late flowering (14/04/11 and later). **(b)** Different flowering duration. The two groups for flowering duration are Medium (14-28 days) and long (greater than 28 days). Error bars represents  $\pm 1$  S.E.

**Table 6(a):** ANOVA table for flowering start date and proportion of infestation

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000787	1	0.000787	0.023477	0.879217	4.159615
Within Groups	1.039026	31	0.033517			
Total	1.039813	32				

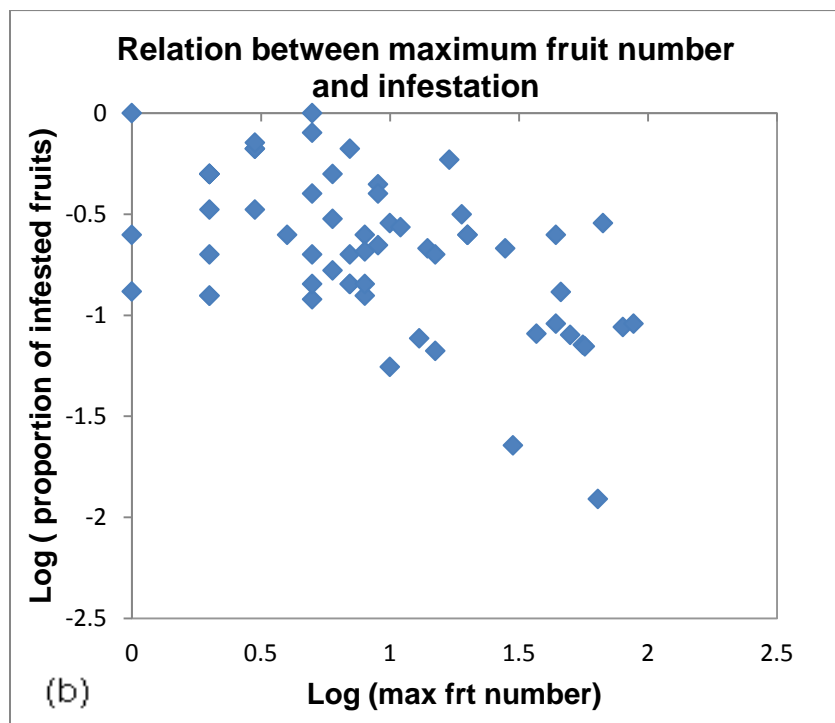
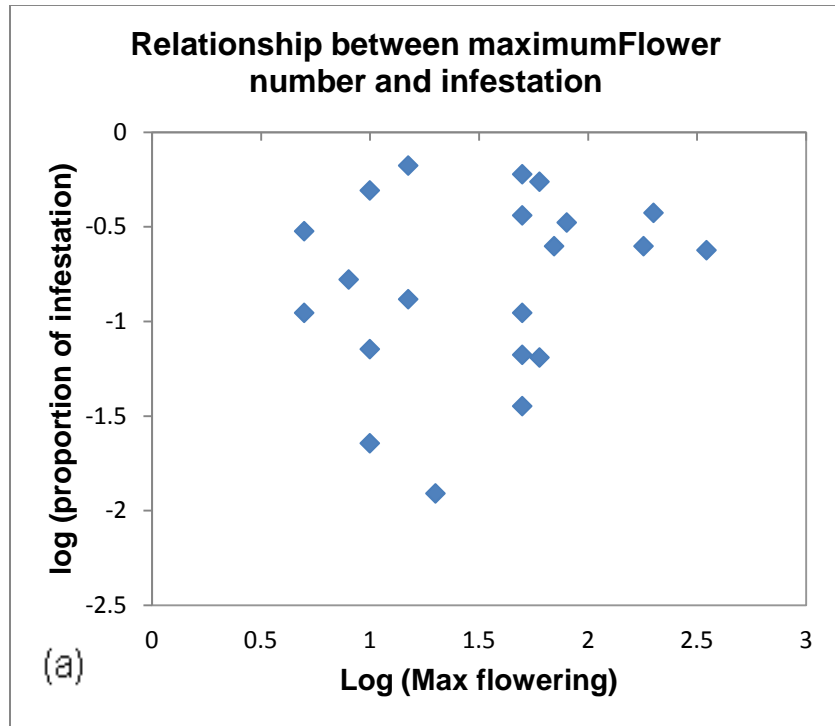
**Table 6(b):** ANOVA table for flowering duration and proportion of infestation

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.074993	2	0.037497	1.998494	0.159403	3.443357
Within Groups	0.412774	22	0.018762			
Total	0.487767	24				

### **Infested fruits and Flower and fruit number**

There was no relationship between maximum flower number and proportion of infested fruits (Fig. 10 (a):  $N=21$ ,  $R= -0.244$ ,  $P= 0.146$ ).

Maximum fruit number had a significant negative relationship with the proportion of infestation (Fig. 10 (b):  $N=60$ ,  $R= -0.544$ ,  $P= <0.0001$ ). As the maximum fruit number increases the proportion of infested fruits decreased.



**Figure 10** (a) Correlation between infested fruits and flower number. Maximum flowering was used as a proxy for total flowers. (b) Correlation between Infested fruits and fruit number.

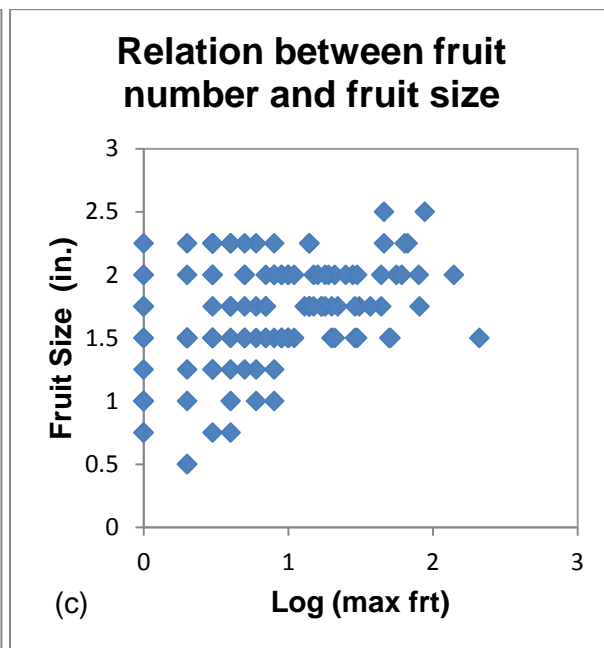
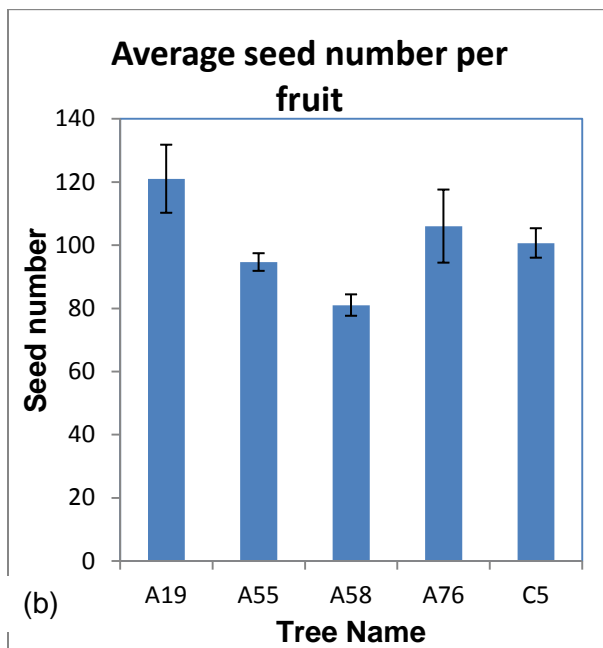
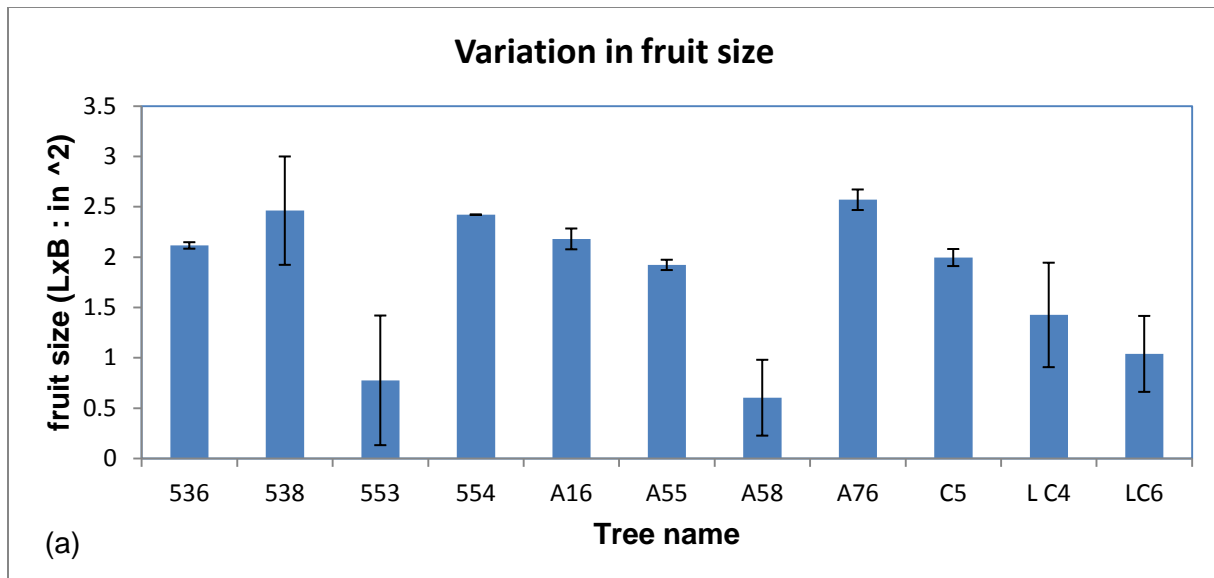
### **Variation in fruit size**

The difference in average fruit size between trees was also examined using the fruits collected from 11 trees. There was a significant difference in the average fruit size between trees (Fig. 11 (a):  $F= 4.405$ ,  $P= 0.00023$ ).

Average seed number per fruit was also significantly different between trees (Fig. 11 (b):  $F= 4.405$ ,  $P= 0.043$ ). Mean seed number per fruit was  $100.12 \pm 4.03$ .

A significant positive correlation was observed between the fruit size and fruit number (Fig. 11 (c):  $N=136$ ,  $R= 0.397$ ,  $P= <0.0001$ ). This relationship was also examined for raw data (not log transformed) and it was also significant ( $N= 136$ ,  $R= 0.241$ ,  $P=0.003$ ).





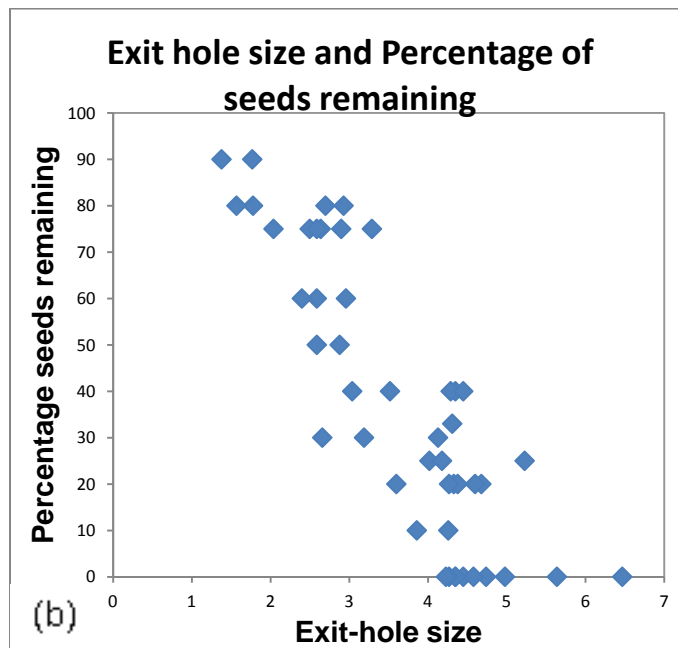
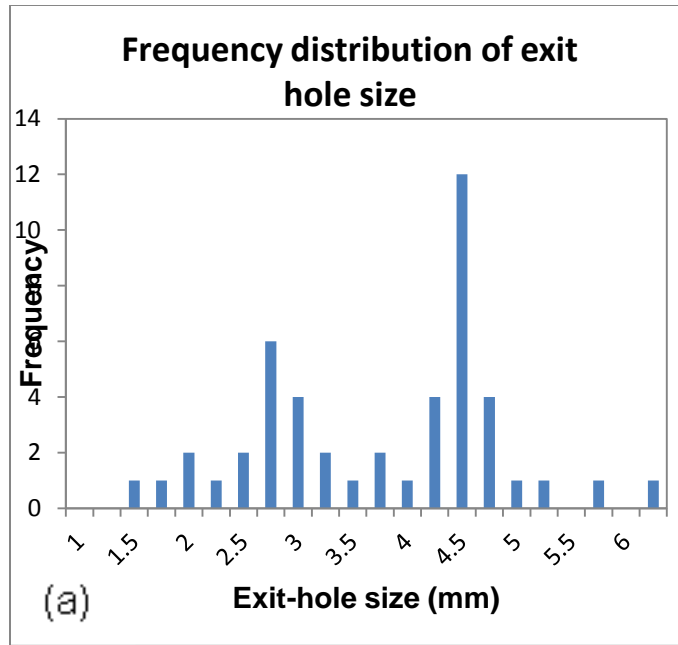
**Figure 11–** (a) Difference in average fruit size per tree. The fruits were collected randomly from 11 trees. L (length) multiplied by B (Breadth) has been taken as a value for fruit size. (b) Average seed number per fruit for different trees. (c) Relationship between fruit size and fruit number. Maximum number of fruits initiated by a tree in the season is compared with the maximum fruit size. Error bars represents  $\pm 1S.E.$

### **Exit holes for infested fruits**

The frequency distribution of exit-hole diameter was studied and found that there is the bimodal distribution of the holes indicating the presence of more than one pre-dispersal predator (Fig. 12 (a)). The exit-holes can be distributed in two groups of diameter size, small ( $< 3.5$ ) and large ( $\geq 3.5$ ) seeds.

Average exit-hole diameter for 'small' group was  $4.46 \pm 0.11$  and 'big' group was  $2.52 \pm 0.12$ .

The exit-hole size was compared with percentage of seeds remaining in the fruits and a significant negative relationship was observed (Fig. 12 (b):  $R = -0.853$ ,  $P = < 0.0001$ ). The difference in percentage seeds were compared for the two groups of small and large seeds using ANOVA and found that large exit hole fruits have less percentage of seeds remaining ( $F = 108.33$ ,  $P = 1.466E-13$ ).



**Figure 12:** (a) Frequency distribution of the exit-hole size of pre-dispersal predators. (b) Relationship between exit-hole size and percentage of seeds remaining.

### **Qualitative comparison for fruiting trees between years**

Individual trees in 2010 and 2011 were compared for number of fruiting trees.

The difference in the number of fruiting and non-fruiting trees in 2010 and 2011 was significant (Chi-square= 237.1522, df =1, P-value= <0.0005).

It shows that there is a high probability that if a tree did not fruit in 2010, it will not fruit in 2011.

If we remove the trees which did not fruit in both the years from the analysis and compare the rest of the trees for fruiting pattern, we get a significant difference (Chi-square = 13, df = 2, P-value= <0.0025).

We observed that the trees which fruited in 2010 had a higher probability of not fruiting in 2011 but trees which did fruit in 2010 had a low chance of fruiting in 2011.

**Table 7:** Fruiting pattern year-wise

<b>Fruiting</b>	YES in 2011	NO in 2011
YES in 2010	44	76
NO in 2010	21	228

### **Quantitative comparison of fruiting in 2010 and 2011**

A quantitative fruiting comparison was done for the trees which fruited in 2010 or 2011 or both. Trees which did not fruit both the years were removed from this analysis as we were looking at the pattern of fruiting between the two years.

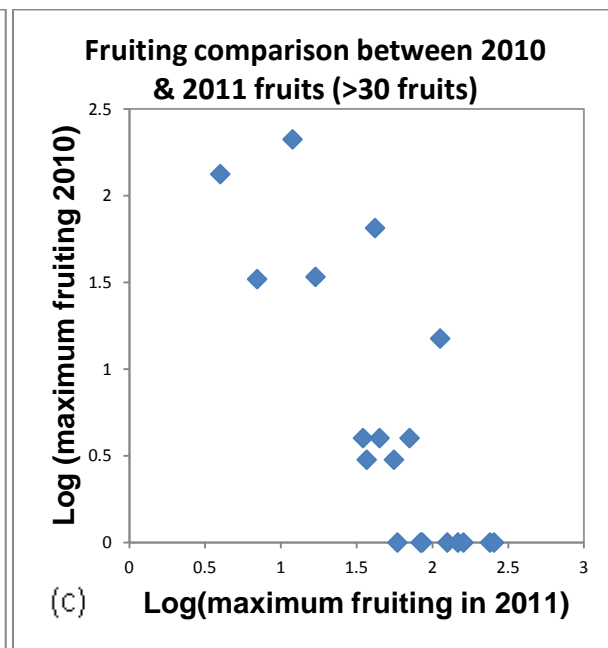
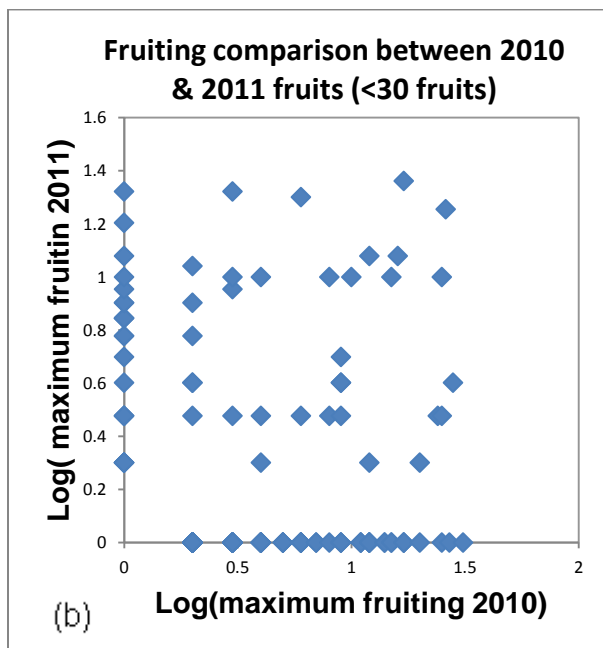
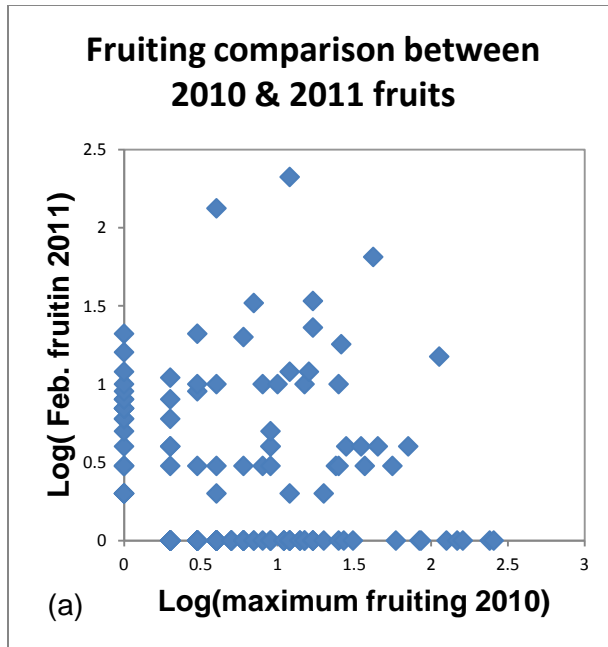
For this analysis the latest fruit number (Feb, 2011) was taken fruiting in 2011, as that can be comparable to the fruiting data of 2010 which was latest in that season.

The relationship between both years' fruiting was not significant when all the trees were taken together (Fig. 13 (a):  $N= 141$ ,  $R = -0.087$ ,  $P = 0.304$ ).

Then the analysis was broken down in two parts, fruit number greater than 30 in either 2010 or 2011 and less than 30 in both the years, as we observed a visible negative relationship with the higher fruit number. So we were checking if the fruit number is higher in any of the season will it affect the fruit number in the other.

The trees with less than 30 fruits were compared and there was a negative correlation (Fig 13 (b):  $N= 122$ ,  $R= -0.185$ ,  $P=.0.041$ ).

Then the trees which had fruit number greater than 30 was compared for between year fruiting and there was negative correlation and it was highly significant (Fig. 13 (c):  $N= 19$ ,  $R=-0.818$ ,  $P= <0.0001$ ).



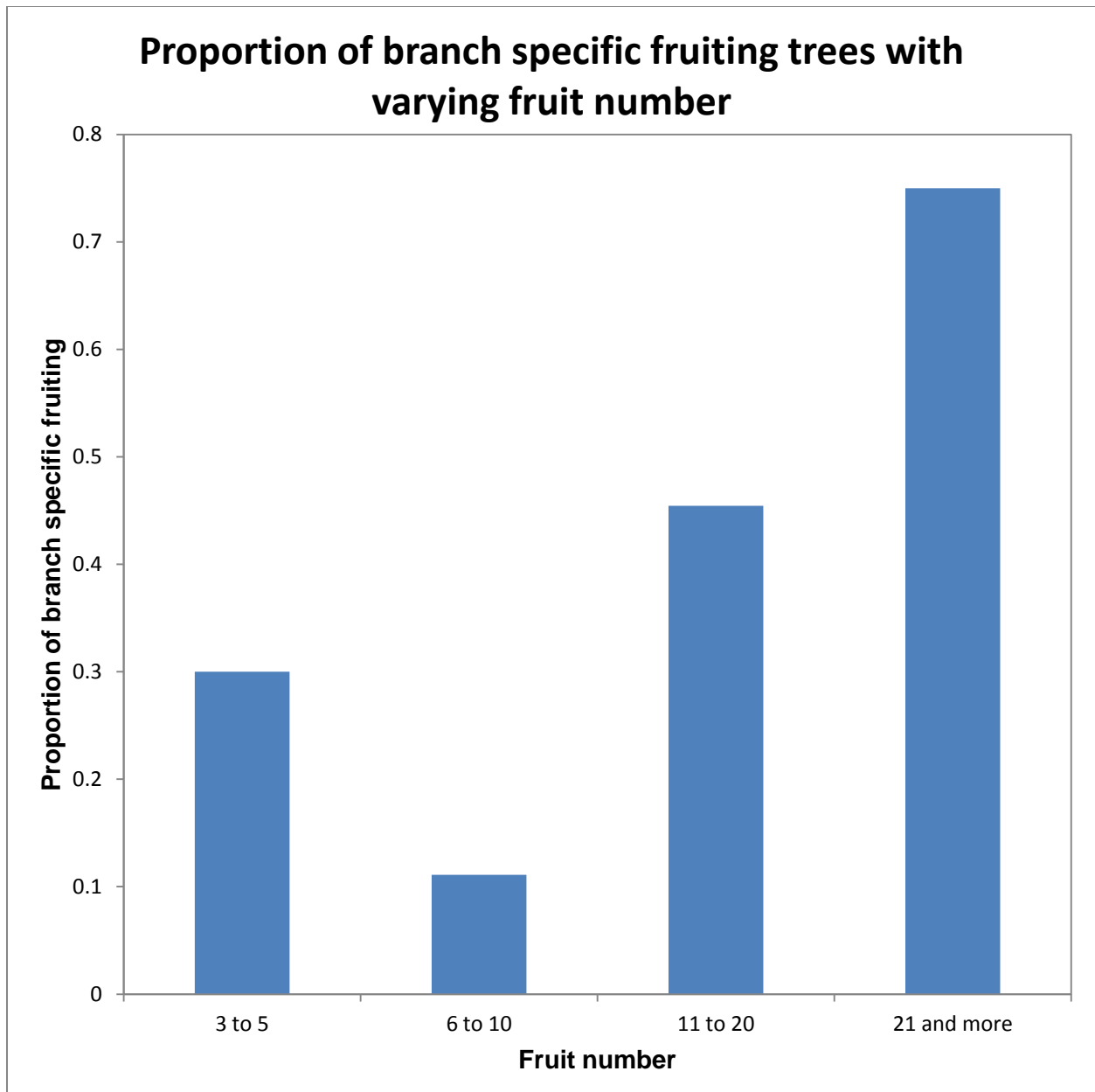
**Figure 13:** Relationship between fruiting in 2010 and 2011. (a) All trees were taken together to see the relationship. As no relationship was found the data was broken into two parts (b) Trees with fruit number <30 and (c) Trees with fruit number > 30. All the trees which were not fruiting in both the years are taken out of this analysis; as we assumed them to be non-fruiting.

### **Branch-specific Fruiting**

Studies show that there is a possibility of presence of IPU's in an individual and different IPU's might have varying resource allocation and fruit number (R. Wyatt, 1982). So, we tested this in our system with a null hypothesis that the fruiting is uniform in the trees.

Total 38 trees were used for this experiment. The trees in this study were distributed in 4 groups (using fruit number) and each individual tree was tested separately for branch-specific fruiting, using chi square test.

The proportion of trees with significant branch-specific fruiting was different for every group. It was found that high fruit number trees tend to have more branch-specificity than low fruiting ones (Fig. 14).



**Figure 14:** The difference in proportion of branch-specific fruiting trees with different fruit number. 38 trees were selected, divided in groups (according to fruit number) and tested for branch-specific fruiting using chi-square test. This figure represents the proportion of trees in each group which show branch-specific fruiting.



## DISCUSSION

Fruit size and number in *Randia* change through the season. Fruit number increased during the monsoon season (July – September). After the end of rains in September, fruit number started declining. Fruit growth was high at the start of the rains and declined slowly (after the end of the monsoon) to reach a maximum.

Average percentage fruit set in *Randia* was calculated to be 0.0195 i.e. ~0.02 (Standard error =  $\pm 0.01$ , N = 64). Earlier studies have shown that there are various ecological correlates of fruit set like mating system (Self-incompatible = 0.332; Self-compatible = 0.691), breeding system (hermaphrodite = 0.394; andromonoecious = 0.579; monoecious = 0.517; dioecious = 0.860), latitude (tropical = 0.386; temperate = 0.510), life form (woody perennial = 0.339, herbaceous = 0.620), fruit type, pollination system etc (Sutherland, 1986a). It was shown that mating system is an important factor that determines fruit set in plants, followed by breeding system. Fruit type (cost of fruit production) and pollination system has very low contribution in determining fruit set (Sutherland, 1986a).

We used site as a proxy of amount of resources present at a given location, as there was a variation in soil depth in all the three sites. So, the working assumption was that, if resources in soil are more the height of the tree will be more and the number of fruits will also be more at that site. When the relationship of site, height and fruit number was examined, we found that tree height was greatest in Hill while fruit number was highest for *Husa* main site. This is contradicting our initial assumption. This may be due to two reasons: firstly, height alone is not related to resources. Height of a tree can be greater due to competition for light. Secondly, resources available at a location can be high but resources available to individual trees need not reflect this due to greater density of individuals in these locations.

Across all sites, we found that taller trees have a greater number of flowers and fruits. The relationship between flower and fruit number will be dependent on their relationship with height of trees. It was observed that if flower number was high, it lowered the maximum fruit number. One explanation can be that if plants devote a high amount of

stored resource for flowering as it is a costly process, then there will be low resources remaining for fruits.

Phenology, flowering date and duration did not affect number of flowers. The number of flowers in a tree was similar for both early and late flowering plants and longer and shorter flowering duration. On the other hand, number of fruits was higher when flowering started late in the season. A likely explanation for this was the higher abundance of pollinators later in the season (D. Barua, unpublished). Increased duration of flowering resulted in lower fruit number. This is against the general understanding that longer flowering trees have greater chances of pollination and the fruit number is high (Elzinga et al., 2007; M. Morgan, 1993; Primack, 1987; Sutherland, 1986a).

Studies show that the fruit number and size have a trade-off in a resource limited environment within species (Sutherland, 1986a) and resource rich environments will favour high number of big fruits (M. Morgan, 1993; Sutherland, 1986b). There was a positive relationship observed between maximum fruit number and maximum fruit size in *Randia*, also the variation in fruit size seem to decrease with increase in fruit number.

In Northern tropics, the percent seed survivorship was found to be ranging from 8.8% to 99.9% (Moles & Westoby, 2003). *Randia* shows a high seed survivorship from pre-dispersal predator of  $83.38 \pm 1.77$  %. The factors which can affect the pre-dispersal predation are flowering phenology (flowering start date, duration), number of flowers (attractiveness of plant), and fruit number (Delph & Sutherland, 1984; Schaik et al., 1993; Stephenson, 1981; Sutherland, 1986a).

As Pre-dispersal predator lays its eggs in the flowers, it takes the cue for egg laying using flowering phenology. The predator starts infecting when the flowering starts and tries to maximize the infestation when flowering is also at its peak. So infestation should vary positively or negatively with flowering time or duration. But in *Randia* no relationship was observed between infestation and flowering start date and duration.

Number of fruits and the proportion of infestation were negatively correlated. It can be explained with the predator satiation hypothesis which states that, as the total number of fruits initiated increases the predator gets satiated and proportion of infested fruits

decreases (Elzinga et al., 2007; Jansen, 1971). But no relationship was observed with number of flowers and proportion of infestation.

Comparing the fruiting across years (2010 & 2011) we found that the trees fruiting in one season can affect the fruiting in next year. We found that if a tree fruits high in one season then it is most likely to have low fruiting next season. It suggests that *Randia* uses previous years' stored resource for flowering. So if the fruit number is higher, it will use up all the resource and will be left with low amount of storage for reproduction in next year and if the fruiting is low in one year then the stored resource may help in improving fruit number in the next year.

It was also observed that fruiting is not uniform across the canopy. There exists branch-specificity to the allocation of resources, hence giving some parts of the tree more resource to grow greater number of fruits than others. This might happen due to the presence of IPU's in a plant. Some IPU's attract more resources from the source than others. Hence we can see a difference in fruiting across different IPU's (Casper & Nisenbaum, 1993; Diggle, 1995; R. Wyatt, 1982).

In future, this study can be improved, by precise census of flowering status and number of flowers. It can help in better identification of flowering start date, end date and duration. It will facilitate in better estimation of cumulative flower number. From the better results, we can improve the values of fruit set, relationship between flowering phenology and other ecological correlates.

Experiments can be done to identify the role of stored resource, like, keeping a tree defoliated for a whole season and testing the fruit set next year. Other experiments can be conducted to identify the mating and breeding system of the species and compatibility. These can help us in making better judgements about the factors affecting fruit set, as the conclusions of the result will change according to the mating or breeding system of *Randia*.

Also, this study can be extended to understand the dispersal patterns and germination of this species, which can give us understanding in the complete reproductive ecology of *Randia*.

## REFERENCES

- Abe, T. (2001). Phenology, display size and fruit-set in an dioecious understory shrub , ( Cornaceae ). *Evolution*, 88(3), 455-461.
- Aizen, M. A., & Harder, L. D. (2007). Expanding the limits of pollen-limitation concept : Effects of pollen quantity and quality. *Ecology*, 88(2), 271-281.
- Andersen, A. N. (1989). How important is seed predation to recruitment in stable populations of long-lived perennials ? *Oecologia*, (81), 310-315.
- Ashman, A. T.-lynn, Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., et al. (2004). Pollen Limitation of Plant Reproduction: Ecological and Evolutionary Causes and Consequences. *Ecology*, 85(9), 2408-2421.
- Augspurger, C. K. (1983). Phenology, Flowering Synchrony, and Fruit Set of Six Neotropical Shrubs. *Biotropica*, 15(4), 257-267.
- Barrett, S. C. H. (1998). The evolution of mating strategies in flowering plants. *Science*, 3(9), 335-341.
- Berry, P. E., & Calvo, R. N. (1991). Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). *Plant systematics and Evolution*, 174, 93-101.
- Brody, A. K. (1997). Effects of Pollinators, Herbivores, and Seed Predators on Flowering Phenology. *Ecology*, 78(6), 1624-1631.
- Burd, M, & Callahan, H. S. (2000). What does the male function hypothesis claim ? *Journal of Evolutionary Biology*, 13, 735-742.
- Burd, Martin. (1994). Bateman's Principle and Plant Reproduction : The Role of Pollen Limitation in Fruit and Seed Set. *New York*, 60(1), 83-139.
- Cariveau, D., Irwin, R. E., Brody, A. K., Sevillano, L., Garcia-Mayeya, & Ohe, A. von der. (2004). Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *OIKOS*, (104), 15-26.
- Casper, B. B., & Nisenbaum, R. A. (1993). Pollen versus resource limitation of seed production. *Current Science*, 65(3), 210-214.
- Chamberlain, J. . N. H. and S. A. . (2007). Ecological and Evolutionary Mechanisms for Low Seed : Ovule Ratios : Need for a Pluralistic Approach? *Ecology*, 88(3), 706-715.

- Crawley, M. (1989). Insect Herbivores and Plant Population Dynamics. *Annual Review of Entomology*, 34(1), 531-564. doi:10.1146/annurev.ento.34.1.531
- Delph, L. F., & Sutherland, S. (1984). On the Importance of Male Fitness in Plants : Patterns of Fruit-Set. *Ecology*, 65(4), 1093-1104.
- Diggle, P. K. (1995). Architectural effects and the interpretation of patterns of Fruit and seed development. *Ecology*, 26(1995), 531-552.
- Ehrlén, J. (1996). Spatiotemporal Variation in Pre-dispersal Seed Predation Intensity. *Oecologia*, 108(4), 708-713.
- Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time : flowering phenology and biotic interactions. *trends in Ecology and Evolution*, 22(8). doi:10.1016/j.tree.2007.05.006
- Feer, F., & Forget, P.-michel. (2002). Spatio-temporal Variations in Post-dispersal Seed Fate. *Biotropica*, 34(4), 555-566.
- Hulme, P. E. (1994). Post-Dispersal Seed Predation in Grassland: Its Magnitude and Sources of Variation. *Journal of Ecology*, 82(3), 645-652.
- Jansen, D. H. (1971). Seed Predation by Animals. *Annual Review of Ecology and Systematics*, 2, 465-492.
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dush, M. R., et al. (2005). Pollen Limitation of plant reproduction and Process. *Annu. Rev. Evol. Syst.*, (36), 467-97. doi:10.1146/annurev.ecolsys.36.102403.115320
- Kudo, G., & Suzuki, S. (2002). Relationships between Flowering Phenology and Fruit-Set of Dwarf Shrubs in Alpine Fellfields in Northern Japan: A Comparison with a Subarctic Heathland in Northern Sweden. *Arctic, Antarctic, and Alpine research*, 34(2), 185-190.
- Moles, a. T., & Westoby, M. (2003). Latitude, seed predation and seed mass. *Journal of Biogeography*, 30(1), 105-128. doi:10.1046/j.1365-2699.2003.00781.x
- Moles, A. T., Warton, D. I., & Westoby, M. (2003). Do Small-Seeded Species Have Higher Survival through Seed Predation than Large-Seeded Species ? DO SMALL-SEEDED SPECIES HAVE HIGHER SURVIVAL THROUGH SEED PREDATION THAN LARGE-SEEDED SPECIES ? *Ecology*, 84(12), 3148-3161.
- Morgan, M. (1993). Fruit to flower ratios and trade-offs in size and number. *Evolutionary Ecology*, (7), 219-232.

- Obeso, J. R. (2002). Cost of reproduction in plants. *New Phytologist*, (139), 321-348.
- Ogler, D. O. W. V., & Alisz, S. U. K. (2001). Sex among the flowers : The distribution of mating systems. *Evolution*, 55(1), 202-204.
- Parachnowitsch, A. L. ., & Christina M. Caruso. (2008). Pre-dispersal Seed Herbivores , Not Pollinators , Exert Selection on Floral Traits via Female Fitness. *Ecology*, 89(7), 1802-1810.
- Primack, R. B. (1987). Relationships among flowers, fruits and seeds. *Annual review of Ecology and Systematics*, 18(1987), 409-430.
- Sabat, A. M., & Ackerman, J. D. (1996). Fruit Set in a Deceptive Orchid : The Effect of Flowering Phenology , Display Size , and Local Floral Abundance. *American Journal of Botany*, 83(9), 1181-1186.
- Schaik, C. P. V., Terborgh, J. W., Wright, S. J., Wright, S. J., Schaik, C. P. V., & Terborgh, J. W. (1993). The phenology of tropical forests : Adaptive Significance and Consequences for Primary Consumers. *Annual Review of Ecology and Systematics*, 24, 353-377.
- Schupp, E. W. (1988). Factors affecting post dispersal seed survival. *Oecologia*, 76, 525-530.
- Shuster, S. M. (2009). Sexual selection and mating systems. *PNAS*, 106, 10009-10016.
- Stephenson, A. G. (1981). Flower and fruit Abortion : Proximate causes and Ultimate functions. *Ecology*, 12(1981), 253-279.
- Stephenson, A. G. (1984). The Cost of Over-initiating Fruit. *American Midland Naturalist*, 112(2), 379-386.
- Sutherland, S. (1986a). Patterns of Fruit-Set : What Controls Fruit-Flower Ratios in Plants ? *Evolution*, 40(1), 117-128.
- Sutherland, S. (1986b). Floral Sex Ratios , Fruit-Set , and Resource Allocation in Plants. *Ecology*, 67(4), 991-1001.
- Wesselingh, R. A. (2007). Pollen Limitation Meets Resource Allocation: Towards a Comprehensive Methodology. *New Phytologist*, 174(1), 26-37. doi:10.1111/j.1469-8137.2007.01997.x
- Wilcock, C., & Neiland, R. (2002). Pollination failure in plants : why it happens and when it matters. *Trends in Plant Science*, 7(6), 270-277.

Wyatt, R. (1982). Inflorescence architecture : How flower number, arrangement and phenology affect pollination and fruit-set. *American journal of Botany*, 69(4), 585-594.

Wyatt, Robert. (1981). The Reproductive Biology of *Asclepias tuberosa*. II. Factors Determining Fruit-Set. *New Phytologist*, 88(2), 375-385.

Zimmerman, M. (1980). Reproduction in *Polemonium* : Pre-Dispersal Seed Predation. *Ecology*, 61(3), 502-506.