The impact of variation in ‘false’ food signalling on the pollination success of *Phacelia campanularia* and *Parnassia palustris.*

Dianne Sutherland

04465845

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Supervisor: Dr. C. C. Wilcock

Contents

1. Abstract

Many flowering plants possess morphological features that appear to mimic food signals, yet their impact on pollination success has rarely been studied. Two plants with such food signals were investigated; *Phacelia campanularia* with white corolla markings and *Parnassia palustris* with five staminodes.

The flowers were experimentally manipulated. In *P. campanularia,* the corolla spots were disguised with paint. In *P. palustris* the staminodes were removed. Both species were exposed to open pollination and visitation rates were compared between the manipulated plants and the control plants. Flowering period, flower production and pollen load were investigated in *P. campanularia.* Reproductive success was assessed from fruit set, seed number per fruit, seed crop and seed mass.

In *P. campanularia* the insect visitation rate was 27% lower when corolla spots were disguised. In *P. palustris* the visitation rate was 29% lower when the staminodes were absent. Visitor numbers varied between pollinator groups and according to feeding preferences. In *P. palustris* the type and number of insect visitors was influenced by differing environmental conditions.

Fewer pollen grains were deposited onto the stigmas of *P. campanularia* when the corolla spots were disguised ($P=0.03$). Lower levels of pollinator movement resulted in insufficient pollen being delivered to initiate fruit set in many flowers. Pollen limitation was the likely cause of the excess flower production (29% greater) in *P. campanularia* treatment plants. Reproductive success was lower in the absence of spots in terms of fruit set, seed mass and seed crop ($P<0.05$). Resource limitation affected seed development in treatments, as plants producing excess and prolonged flowering appeared to exhaust their resources, leaving insufficient time for fruit maturation.

In *P. Palusrtis*, although insect visitation was lower in treatment plants, fruit set was unaffected by staminode removal, probably because pollen delivery reached sufficient threshold levels to initiate fruit set. However, fruit size and seed mass were lower when staminodes were absent ($P<0.05$). Pollen clogging was the likely cause of reduced fruit size/mass in treatment flowers. The absence of the staminodes may have resulted in self or non-compatible pollen, from non-pollinating insects, coming into contact with the stigma, thus limiting the number of ovules fertilised.

These findings support the hypothesis that pollinators exhibit a preference for flowers with markings or features that mimic pollen or nectar and that these food signals can significantly increase reproductive success and therefore may be evolutionary selective factors in plant reproductive biology**.**

2. Introduction

2.1 Plant pollinator interactions.

Christian Konrad Sprengel first described the mutualistic interactions between plants and their pollinators in 1793. In this relationship a pollinator receives food when visiting a flower, usually in the form of pollen or nectar, and in return the plant has its pollen carried from one flower to the stigma of another, which, in turn facilitates cross fertilisation and genetic diversity**.** However, in order for the plant to attract the pollinator and ensure constancy it has to successfully advertise its food rewards to visitors (Proctor *et al*. 1996).

The range of diversity present in flowers reflects the evolutionary modification of just a few floral forms (Walker-Larson and Harder, 2000). Fossil evidence has revealed that angiosperm-insect interactions appeared early in the evolution of flowering plants, dating as far back as the Cretaceous period (142 mya) (Ren, 1998, Sun *et al*. 1998.). Insect choice for particular floral features during the mid–Cretaceous period is viewed as an important facilitator of angiosperm diversification (Kearns and Inouye, 1997). Pollen is thought to be the original attractant offered to insects, providing both a rich nutritive source and a visual stimulant (Osche, 1979.) Nectar was a later development in floral evolution and became the most frequent reward offered to insects (Cruden *et al.* 1983, Proctor *et al.* 1996). This development was advantageous as nectar is produced as a by-product of plant metabolism and as such is produced at a lower energetic cost than pollen. For most adult winged insects nectar provides the main food source, carbohydrate. Although nectar provides sufficient food reward for many insects, bees have exerted strong selection for protein rich pollen flowers. They are the most important flower visitors because in addition to their feeding requirements they also require pollen for their larvae to develop. Subsequently they visit flowers more frequently than any other insects. (Proctor *et al*.1996).

Many adaptive floral features that attract pollinators to their food source have been selected for by insects, such features include flower shape, colour, scent and markings. Similarly flowers have also shaped the evolution of insect collecting organs, sensory stimulation and learning behaviour (Barth, 1985). Two factors, interspecific competition between plants and insect constancy have been proposed as the main selective forces in the divergence of floral forms (Ostler and Harper, 1978). These relationships and the resulting adaptations have enabled angiosperms to become the most globally dominant plants (Dilcher, 2000).

Today it is estimated that there are 250,000 flowering plant species with up to 67% being dependent on insects for pollination (Kearns, and Inouye, 1997). Up to 20% of the 800 000 species of insects are dependent on pollen or nectar for food at some point in their life history (Barth, 1985).

2.2 Insect perception of floral traits and foraging preferences.

Naïve insects must be able initially to find food and then to relocate the flowers that are the most rewarding, ignoring flowers which are not profitable (Chittka and Thomson, 2005). Food sources should be suitable in terms of flower handling if optimal foraging is to be achieved (MacArthur and Pianka, 1966). Hymenopteran visitors require food for themselves and their larvae, as they often travel long distances from the nest to find food, they must make quick decisions during foraging. As a consequence of optimality, specialisation may occur between a flower and its pollinator (Chittka and Thomson, 2005). Dipteran visitors, especially Syrphids, are more commonly generalist foragers, they feed on a wide variety of plants, and are the most common visitors to flowers they feed on both pollen and nectar depending on the ease of accessibility determined by the flower shape (Kearns, 2001). Specialist and generalist foragers may adopt different strategies or switch foraging methods depending on environmental variables such as patch quality and size, fragmentation, temperature or competition within a patch (Charnov, 1976). Evidence suggests that memory involved in decision making is complex and the mechanisms employed by pollinators operate at different levels by using innate preferences and learned experiences (Proctor *et al*. 1996).

In 1914 Karl von Frisch's (cited in Proctor *et al*. 1996) early bee training experiments, it was first demonstrated that bees were able to distinguish between colours. Work by Alfred Kühn and Robert Pohl in 1921(cited in Barth 1985) revealed the wavelength spectra visible to the bee, although similar to human vision, is shifted but shifted into the shorter wavelength UV spectrum and away from red at the other end of the spectrum. The basic *bauplan*, UV-blue-green-trichromatic vision was a later discovery the origin of which dates back to the Devonian period (Autrum and Zwehl, 1964, cited in Barth 1985). Although colour receptors do differ, the majority of insects also have identical sets of colour receptors (Briscoe and Chittka, 2001). It can therefore be assumed that the visual signals that attract pollinators are similar for many insects.

Large floral structures, colours, shapes and odour have been shown to attract visitors from long distances and during early learning experiences (1980, Proctor *et al.* 1996, Chittka and

Thomson, 2005). The UV spectrum markings have been demonstrated as important nectar guides that direct the visitor and alert them to floral shape and potential landing sites (Barth 1985, Proctor *et al*. 1996) Petal markings and internal floral features, or even prey, act as short-range cues to insects. Insects have also been shown to display strong preferences for flowers with more than one colour and colours that are sharply contrasting (Heuschen *et al.* 2005). In addition long and short-range visual cues have been shown to complement each other in flower signalling (Dafni and Kevan, 1996).

2.3 Flower Signalling, Mimicry and Deception.

The classic explanation of mutualism provides us with a useful model for the study of co-evolution (Boucher, 1982). This explanation predicts that floral advertising should give a true reflection of the food rewards present in a flower and that a pollinator's energetic demands should lead to selection for floral cues, which are predictive of food rewards. While this is true in many plant pollinator interactions there are many plants that appear to exploit insect sensory perception, these plants often deceive the pollinator into missing out on the most profitable rewards. (Basolo, 1995, Johnston 1996, Heiling and Herberstein 2004). Many authors now recognise that mutualistic interactions are likely to be reciprocal exploitations where each participant aims is to increase the benefits and reduce the costs of the interaction (Thompson, 1982, Dufay and Anstett, 2003). Selection pressures, such as limited resources, pollinator and pollen limitation are variable both temporally and spatially because environments are continually changing, as a result of these selection pressures, competition for pollinators may occur both within and between species, (Kay, 1976, Chittka and Thomson, 2005). Plant fitness is achieved by pollen donation and fruit maturation. (Sutherland, 1987) Consequently any false floral adaptations that are perceived as food signals may improve insect visitation and pollen donation, which in turn, may increase plant fitness. This type of selection has resulted in the evolution of 'cheaters' (Dafni, 1984, Anderson *et al.,* 2005).

The phenomenon of 'cheating' is well described in zoology in the form of mimicry. In animals mimicry is frequently used as a classic example of adaptation through natural selection. Many insects adopt the appearance of another unrelated, but toxic species, which confers an adaptive advantage at a lower energy cost than that incurred by the model. The overall effect is an increase in fitness for the mimic that would not otherwise occur without the presence of the model. There are broadly two types of mimicry described within zoology, Batesian and

Mullerian, both of which act as predator evasion adaptations. In flowers the situation differs, as the effect is not to deter a predator but to attract a pollinator. Batesian mimics offer no reward and as such are subject to negative frequency dependence, the mimic must remain at low density in order to gain the benefits of the model. In Müllerian mimicry both the model and the mimic offer the pollinator a reward which benefits both species (positive frequency dependent) and this is an example of two different species converging on similar phenotypes (Roy and Widmer, 1999).

Several authors have discussed various forms of deception in flowers, most notably in Orchidaceae, which generally exhibit Batesian mimicry (Dafni, 1984, Roy and Widmer, 1999, Johnson, 2000, Anderson *et al.*, 2005) far fewer few have discussed floral Mullerian mimicry (Roy and Widmer, 1999). Dafni (1984) has described over fifty examples but suggests the phenomena is much more widespread than is generally accepted. Mimicry of pollen and anthers within the same species has only recently been presented in scientific literature. First suggested by Osche in 1983, the pollen mimicry hypothesis suggests that a similarity exists between the colour of pollen and a small sized, similar coloured central area of the flower. This hypothesis has rarely been tested and only performed using artificial flowers and environments. A recent study by Heuschen *et al*. (2005) found that bumblebees showed a preference for artificial flowers displaying central colour patterns that closely matched the spectral reflectance of the pollen. The same study also found that in spectral analysis of 162 species of flowers, that the colour loci of the central pattern markings were clustered with in the same area as that of the pollen colour. The author’s findings support the pollen mimicry hypothesis and suggest that bumblebees possess innate preferences for visual pollen signals and that pollen mimicry forms a generalised mimicry system in angiosperms.

The significance and reproductive success of flowers exhibiting food mimicry signals has only been studied once in the field and the authors focussed only on the importance of functionless stamens in *Parnassia palustris* (Sandvik and Totland, 2003). This study found that when staminodes were removed, the numbers of insect visitors was always reduced, but found that the mode of pollination differed at two separate sites. They did not consider the roles of different insect visitors or temperature at the sites in this study but suggested that thermal heating was facilitated by staminodes and may therefore influence visitation.

Staminodes are found in many flowers across a broad range of taxonomic groups. They are reduced stamens that have lost their primary function of pollen production. Examples can be found in *Copis trifolia and Penstemon* spp (Walker-Larson and Harder, 2000) and also in heterostylus flowers such as *Commelina colestris* and *Commelina dianthifolia* which as pollen flowers rely on visual cues to attract pollinators by possessing both cryptically coloured stamens and brightly coloured pollen mimicking stamens which are sterile but attract insects (Hrycran and Davies, 2005).

These vestigial features may have been retained because they mimic the appearance of a food reward, the real food reward lies at the base of the staminode and encourages the visitor to explore the flower.

Spots, highly contrasting markings and staminodes are present in many flowers and many potential examples of food mimicry may therefore exist in nature. Interspecific competition between species sharing the same habitat and pollinators may lead to preferences for species with enhanced food signals (Chittka, 2005). Intraspecific variation in flowers is common in many species and it has been shown that some insects prefer one colour morph in preference to another (Kay, 1976). Pollinators may then show a preference for a different morph which has food signals present and this variant may increase in frequency because it successfully deceives the pollinator by enhancing its own reward (Stanton, 1987, Chittka, 2005). Forms without food signals would be expected to suffer lower levels of pollination, pollen limitation and therefore lower fruit set, seed set and seed number (Waites and Agren, 2004). Under this type of insect choice, and particularly where self-pollination is limited, variants without food signals may decline in number. In addition plants may respond to pollen limitation and the resulting low fruit set by increasing or extending flowering (male pollen donation). According to Bateman’s principle (1948) male function will be limited by availability of ovules whereas female function is limited by resources. However these factors are not mutually exclusive and increased flowering may lead to resource limitation particularly in a non-selfing annual plant where time limited reproduction is also a consideration (Johnston, 1991, Snell and Aarrssen, 2005).

Food mimicry may therefore be generalised form of signalling which is widely distributed taxonomically and manifested in many forms and these forms may exploit both the generalist and specialist foragers.

2.4 Aims of this study

In this study *Phacelia campanularia* corolla spots and *Parnassia palustris* staminodes are experimentally altered to investigate whether their presence exploits insect sensory mechanisms by enhancing their food rewards with false food signals. A full assessment of pollination and the subsequent reproductive success will be investigated by examining insect choice for flowers with manipulated food signals; i) in a garden environment for *Phacelia campanularia* and ii) in a field environment for *Parnassia palustris.*

Experimental alteration of false food signals will be carried out. In *P. campanularia* white markings on the petals will be disguised and in *P. palustris* removal of the staminodes will be undertaken to test whether:

1. Insect visitation rates are differ between plants with false food signals compared to plants which have had their false food signals manipulated.

2. Whether types and /or numbers of insect types differ between control and treatment plants.

3. Whether reproductive success differs between control and treatment plants

4. Whether changes in reproductive success have an impact on flowering period and flower production in Phacelia *campanularia*

5. Whether staminodes attract visitors by facilitating thermal heating. As proposed by Sandvik and Totland, 2003.

3. Materials and methods

3.1 Two study plants were chosen for this study: *Phacelia campanularia* and *Parnassia palustris*.

Figure 3.1.1 *Phacelia campanularia*

3.1.1 *Phacelia campanularia* A. Gray, (Hydrophyllaceae), is an annual herb that is native to the Mojave Desert in North America. Flowers are blue, campanulate, with five petals each bearing five stamens with white spherical anthers. Five white spots are present on the blue petals. *P. campanularia* produces many short-lived flowers during its long flowering period. Flowers are hermaphrodite and protandrous. (See figure 3.1.1).

3.1.2 *Parnassia palustris* L. (Parnassiaceae) is a perennial herb that is native to Britain and northern Europe. The white flowers are solitary and disc shaped. Each flower has five staminodes, five petals and five anthers. The staminodes have a nectar-secreting base with glistening honey-like globules at their tips. Flowers are hermaphrodites and protandrous. The thick stamens open in sequence, and this extends the male phase of the flower. (See figure 3.1.2).

Figure 3.1.2 *Parnassia palustris*

3.2 Study 1 . *Phacelia campanularia* experimental design to determine if spots act as false food signals to insect visitors.

Phacelia campanularia seeds were obtained from Chiltern Seeds (Cumbria, UK). Seeds were sown at the Cruickshank greenhouse, Aberdeen, on 9th May 2005. Following germination plants were transported to the observation site at a coastal garden site in Macduff, Aberdeenshire. Plants were potted into 1.5 litre pots using multipurpose compost. A second batch of seeds was sown on 1st July for later greenhouse investigations.

Twenty four treatment plants were randomly selected. Upon opening every flower had the five white corolla spots painted over with Windsor & Newton acrylic paint, Ultramarine blue, series 1, permanence A. A size 1 sable hairbrush was used.

Equal numbers of plants were used in the control group. Paint was applied to all flowers in the same manner, but onto the blue part of the petal (in between the white spots) to balance any affects of the paint to insect visitors (see figure 4.1.1). Both treatment and control plants were photographed with a UV filter and under a UV lamp to detect whether the paint was absorbing or reflecting UV light differently from the rest of the petal.

Following the application of paint, all flowers were tagged below the sepals on the pedical with a small piece of cotton thread. This enabled the identification of control group flowers that had already been painted**.** The same thread was applied to treatment plants to balance any effects.

3.3 Observation of insect visitors.

Flowers were observed for insect visitors from mid July to the end of September. Control and treatment plants were arranged in two random blocks. Insect visits were recorded during a two-hour period each day at between 1200 and 1600 hrs. Insects were identified using Chinery (2005) and Edwards and Jenner, (2005). Insect visitation rates were recorded for all insect families. A total period of 42 hours of observation was recorded.

3.4 Flowering phenology and breeding system

Flower phases were recorded by photography and illustration to observe the male and female phases of the flower. To determine the mode of pollination in *P. campanularia* plants were pollinated with out-cross or self-pollen, fruit set was then compared between groups. Glasshouse hand pollinations were performed using an excess of cross pollen on five separate plants (total of 25 cross-pollinations). The same procedure was carried out using self pollen.

Donor pollen was selected from early male phase flowers immediately following anthesis to ensure fresh viable pollen. Pollen was brushed onto receptive stigmas with a size 3 sable paintbrush. Receptive styles were selected to receive pollen. Flowers were then bagged using lightweight horticultural fleece size 5cm x 4cm to prevent any further pollen import

3.5 Flowering period and flower production

The flowering period was recorded for each plant from appearance of first flower to death of last flower. Total flower production per plant was obtained from five control and five treatment plants from total flower and fruit production.

3.6 Pollen load

Six intact stigmas were collected from the dried fruits of control and treatment plants and examined using microscopy at x 40 magnification. Stigmas were stained using Cotton blue lactol phenol, and left to soak for one day prior to examination. Pollen grains present on the stigma were counted to the nearest five.

3.7 Reproductive success

Fruit % was calculated from total fruit and flower production from five control and five treatment plants.

Seed number and mass per fruit was calculated from fifty fruits, which were randomly selected from 10 control and 10 treatment plants. The number of seeds present in each fruit capsule was counted. Seed mass was measured by removing all the seeds from the fruit capsules and weighing to three decimal places. Mean seed mass per seed was then calculated by dividing the total mass by the seed number per capsule.

Seed crop per plant was calculated by removing all the seeds from six control and six treatment plants.

To test for resource limitation, the number of seeds produced per fruit and the seed mass were measured from plants that were either open pollinated (treatment and control plants) or artificially pollinated (self and outcross), (as described in section 3.4). Seeds were removed from eight fruits, which were selected from two plants from each of the four groups.

3.8 Study 2. *Parnassia palustris* field study at Gardenstown, experimental design to determine if staminodes act as false food signals to visitors.

The study site was chosen at Gardenstown (Grid ref: NJ7964) where a large population (> 1000 plants) of *P. palustris* exists. Fieldwork was carried out from mid July to end of September.

Two observation areas were randomly selected within the main population site. Within each site 30 flowers were randomly selected and these flowers were used as the treatment and control groups. Treatment flowers were manipulated at the bud stage by having their staminode tips carefully removed with small scissors, but leaving the nectaries intact. The treatment plants were identifiable by attaching pink plastic tubing 1mm in diameter around the base of the stem, this tubing was hidden in the vegetation to avoid any influence on insect. Control flowers were selected at the same bud stage; these flowers were left intact and tagged with blue plastic tubing.

To test for self-pollination 12 flowers were randomly selected at the site and bagged using lightweight horticultural fleece. Bags were approximately 15cm high x 10 cm wide and were secured to the ground by stitching to the grass

3.9 Observation of insect visitors

Ttreatment and control flowers were exposed to open pollination and observed for insect visitation during 10 minutes bouts between the hours of 1200 and 1500 hrs. A total of 960 minutes observation was recorded (12 bouts per session over 8 separate periods).

A range of insects was collected from flowers using collecting jars; the insects were then frozen. Identification of insects to family was made using Chinery (2005).

Because modes pollination in *P. palustris* have previously been shown to differ under different environmental variables (Sandvik and Totland 2003), insect visitors were also recorded under different weather conditions as follows: i) dull and cool, ii) dull and warm or iii) warm and sunny.

3.10 Reproductive success.

Fifteen treatment and control flowers were randomly selected following death or maturing of fruits to test for fruit set %, seed size (length and width) and seed mass. The dead flower head or ripe fruits were harvested and compared. Seed size was crudely measured using a metal ruler to the nearest 0.5mm, Seed size has previously been shown to be highly correlated with seed number and so seeds were not counted due to their small size and high number (Sandvik and Totland, 2003). Seeds mass was measured using digital scales to three decimal places.

3.11 Floral Heating

The interior temperature of flowers were compared to ambient temperature to establish if thermal heating was a contributory factor in attracting insects (as suggested by Sandvik and Totland, 2003). Two unsheathed 10k micro thermomister probes connected to a data logger. Temperature readings were logged every 10 minutes. The probes were inserted next to base of the nectaries in both treatment and control plants with the second probe placed nearby the flower to record the air temperature. Data was recorded between 8.00am and 18pm on seven separate days, weather conditions were also noted as cool and dull, warm and dull or sunny and warm. Temperature was also compared between male and female phase flowers in both treatment and controls for 20 separate data readings and on six male and six female flowers.

3.12 Data analysis

Statistical tests were performed using Microsoft EXCEL and Minitab software. All data was tested for normality and equal variance. For comparison of control and treatment populations, a Student t-test was used. Multiple comparisons were analysed using ANOVA. Pairwise comparisons between hand pollination, control and treatment plant were carried out using LSD (adjusted for Bonferroni's method).

Fruit set percentages were analysed for comparison of proportion using Chi- squared tests with Yate's correction applied.

Non parametric tests were used where data was not normal and could not be normalised, in these cases a Mann -Whitney U test was performed.

4. Results

Study 1. *Phacelia campanularia*.

4.1 Floral UV reflection patterns

Treatment flowers, with corolla spots disguised with paint, and control flowers with the same paint applied between their spots (**fig. 4.1.1**) showed no difference in UV reflectance when photographed under a UV filter (not shown) and under a UV lamp (**fig 4.1.2**). Indicating that no modification in UV patterns (observable to visitors) was caused by the addition of blue paint to the petals.

Figure 4.1.1 Control (top left), treatment (bottom) and natural flowers (top right) photographed in natural light.

Figure 4.1.2 The same flowers as above photographed under a UV lamp. No absorption or reflection of UV light was detected from the paint.

4.2 Floral phenology and breeding system.

Within the life cycle of a *P. campanularia* flower the period during which pollination can occur is short (approx. 2 days). Initially anthers appear large and a spread out and the stigma is small and unreceptive. The stigma then grows above anthers, opens and becomes receptive (**fig. 4.2.1**). The maximum display of spots and anthers is observed during the receptive flower phase and gives the impression of more pollen than is actually present. Spots fades with pollen colour (**fig. 4.2.2, 4.2.3 and 4.2.4**).

Figure 4.2.1 Male and female flower phases. Figure on the left shows spread out anthers and unreceptive stigma. Figure on the right shows female flower phase, anthers have moved towards the centre the stigma has grown above and is receptive. Pollen matures before stigma and this reduces the risk of self-pollination.

Figure 4.2.2 Male flower phase, day one.

Figure 4.2.3 Female flower phase, day two.

Figure 4. 2. 4 Flower day 3 onwards.

4. 2 Floral phenology and breeding system (cont.)

The glass house investigation into the breeding system of *Phacelia campanularia* revealed that the plant was largely dependent on cross pollination with 64% of attempted cross pollinations being successful and only 8% of attempted self pollinations setting fruit (**table 4.1**).

Table 4.1. Self-pollination and out crossing experiments in *P. campanularia.* Attempted pollination's performed on 25 flowers, from 5 separate plants, for each mode of pollination, revealed that the plant is predominantly cross-pollinated.

Mode of pollination	Number of attempted pollinations (flowers)	Number of fruit set
Self	25	2
Out cross	25	16

4.3 Effect of corolla spot removal on frequency of insect visits

Treatment plants, with their corolla spots disguised, received 27% fewer visits from insects than the control plant flowers. Visits were significantly lower at P = 0.004 (t-test) (**fig. 4.3**).

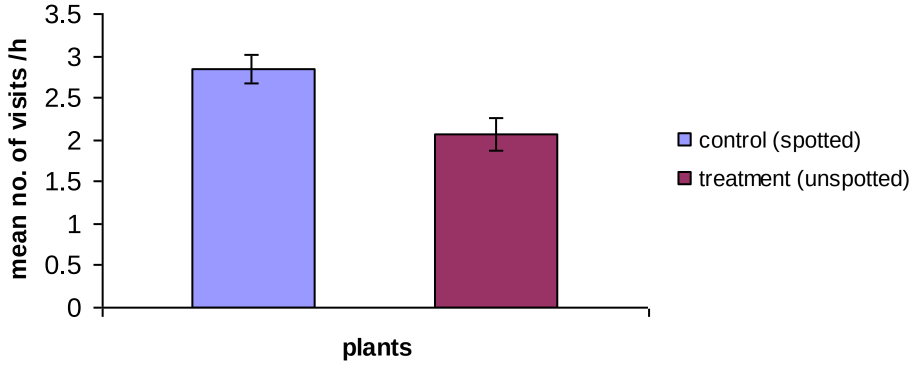


Figure 4.3. Total number of visitors to *P. campanularia* plants. The numbers of insect visits were significantly lower when the white corolla spots were disguised P = 0.004 (t-test). Control plants (n=24) received 2.833 ± 0.165 visits per hour and treatment plants (n=24) received 2.071 ± 0.19 visits per hour (Bars show standard error).

4.4 Types of insect visitors.

Visitor types were identified as follows: 1., Bombus (Hymenoptera) species comprising of *B. terrestris* and *B. lucorum*. 2. Syrphidae (Diptera) species; *Episyrphus balteatus* and *Melanostoma Scalare* 3. *Apis mellifera*. Syphids were observed to feed entirely on pollen, whereas Bombus species fed on pollen and nectar.

There were significant differences in visitation rates between control and treatment flowers for both Bombus and Syrphids. Significantly lower rates were recorded for both groups in treatment plants. Bombus visits were fewer at P = 0.046 (t-test). Syrphid visits were fewer at P = 0.02 (t-test). Apis visits were too low to analyse data and are not considered further. (**fig. 4.4**).

4.4 Types of insect visitors (cont.)

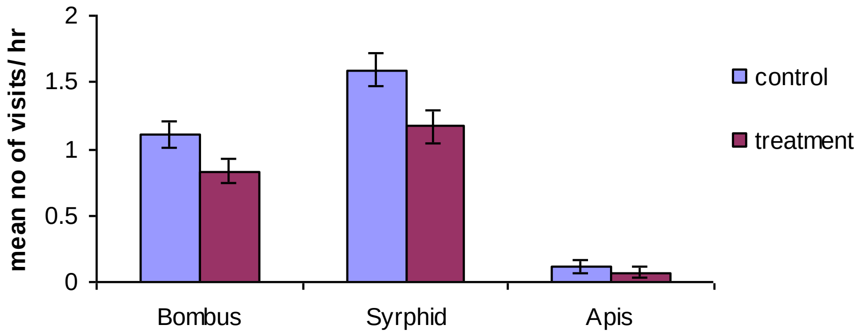
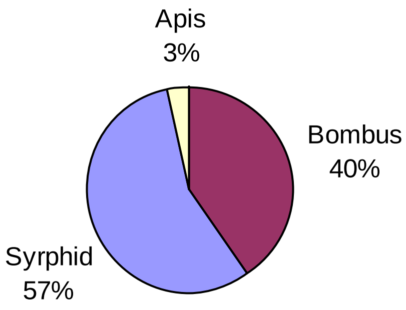


Figure 4. 4 Visitor types to *P. campanularia* control and treatment plants. Bombus and Syrphid visits were significantly lower to treatment plants, P = 0.046 and P= 0.02 (t-test) respectfully. The mean number of visits per hour (± standard error) were as follows: Bombus visits to control plants = 1.111 ± 0.095, treatment plants = 0.833 ± 0.095. Syrphid visits to control plants = 1.595 ± 0.13, treatment plants = 1.116 ± 0.125. Apis control = 0.119 ± 0.05. Apis treatment = 0.071 ± 0.04. Bars show standard error. n=24 for control and treatment plants).

4.5 Variation in visitor spectrum

The spectrum of insect visitors to control and treatment plants were similar (**fig. 4.5.1 and 4.5.2**). The majority of visitors were Syphids spp. and Bombus spp., *Apis mellifera* only accounted for a small percentage of visits**.**



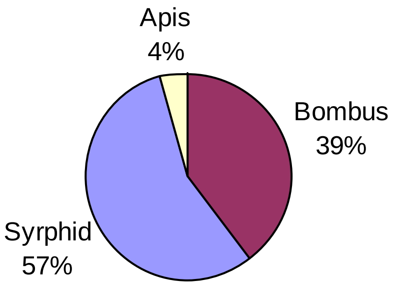


Figure 4.5.1 Visitor spectrum for control flowers

Figure 4.5.2 Visitor spectrum for treatment flowers

4.6 Flowering period and flower production.

The flowering period for each plant was recorded from first flower appearance to last flower dying. The control plants flowering period ranged from between 34 and up to 72 days. Treatment plants flowered for between 47 and 83 days**.**

Treatment plants flowered for a longer period and produced more flowers than control plants P= 0.0046 (t-test) P = 0.018 (t-test) respectfully (**table 4.2**).

Table 4.2 Mean flowering period and flower production. Control plants produced significantly more flowers per plant than treatment flowers, P = 0.018 (t-test). Mean flower production was also significantly greater in treatment plants P = 0.0046 (t-test) (± shows standard error).

Plants	**flowering period(days)**	**flower production per plant**
Control (spotted)	**61.41 ± 1.902**	**46.7 ± 3.85**
Treatment (unspotted)	**68.58 ± 2.052**	**68.5 ± 6.33**
P value	**0.0046**	**0.018**

4.7 Pollen Load

The pollen load present on the treatment stigmas was less than on the control plant stigmas (P= 0.03, t-test) (**fig. 4.7.1)**. Microscopy showed that control stigmas had a large number of pollen grains present, treatment plants had 45% fewer pollen grains **(figs. 4.7.2 and 4.7.3).** A large number of either inviable or other types of pollen also appeared to be present on the treatment stigmas (not shown).

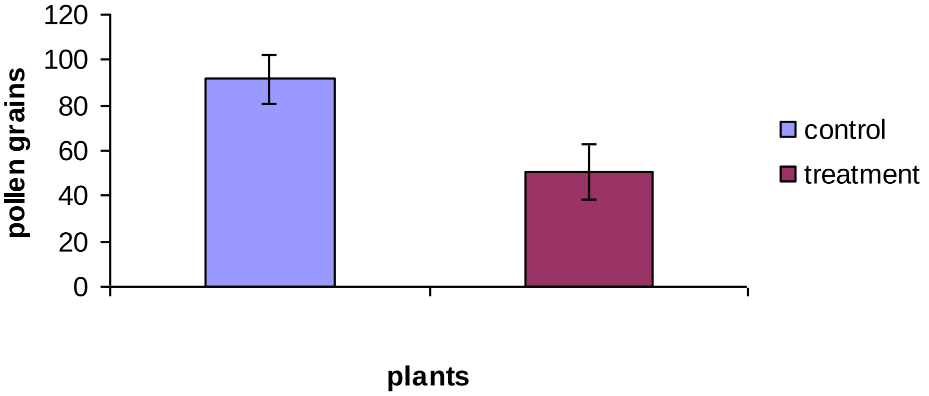


Figure 4.7.1 Pollen load. Mean pollen load per stigma (n=6) was significantly lower in treatment plants than in control plants, P=0.03(t-test). The mean number (± CI) of pollen grains found on the control stigmas was 91.6 ± 10.93, pollen present on treatment stigmas was 50.83 ± 11.93.

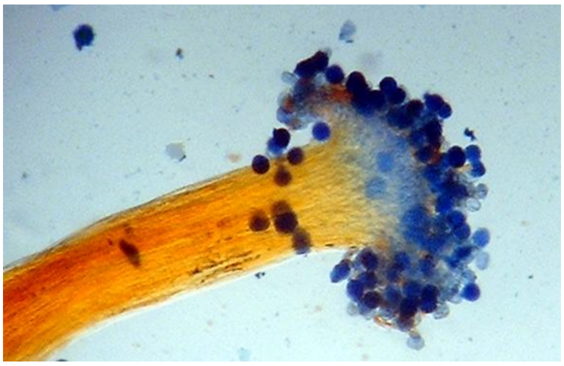


Figure 4.7.2 Control stigma pollen load. Stigma. Shown at x 40 magnification, shows stained (blue) pollen grains. (Image shows only one half of bifid stigma).

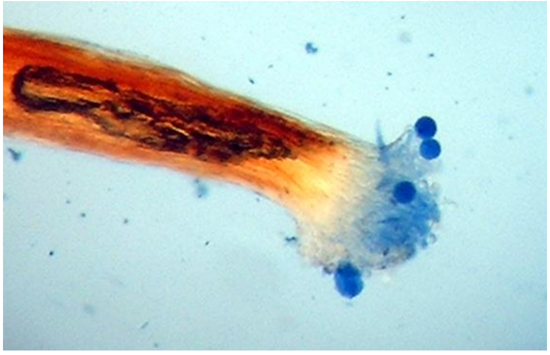


Figure 4.7.3 Treatment stigma pollen load. Stigma of treatment plant at x 40 magnification showing a very low amount of pollen grains (stained blue). (Image shows only one half of bifid stigma).

4.8 Reproductive Success in *P.campanularia*

4.8.1 Effect of corolla spot removal on fruit Set

Treated plants set proportionately fewer fruit than controls. Fruit set was significantly lower in treatment plants, a Chi squared test for comparison of proportion was performed, with Yates correction, $X^2_1 = 5.844$, $P < 0.05$. Control plants produced 54% fruit and treatment plants produced 34% fruit **(fig. 4.8.1).**

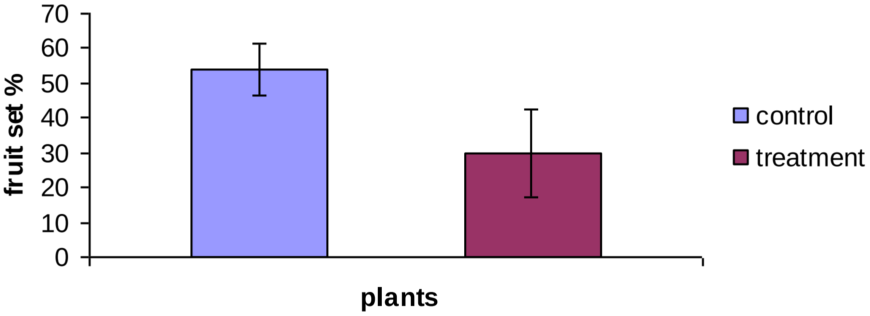


Figure 4.8.1 Fruit set %. Significantly less fruit was set in treatment plants when compared to control plants. Control plants (n = 6) produced 54% of fruit, treatment plants (n=6) produced 34% fruit. A Chi squared test for comparison of proportion was performed, with Yates correction, X21 = 5.844, P< 0.05. Error bars show 95% CI for fruit set per plant (error 12.42 and 7.55 fruit per plant respectfully).

4.8.2 Effect of corolla spot removal on seed number and mass

Fruits from control plants produced significantly more seeds per fruit than treated plants (P = 0.0001 (t-test). **(fig. 4.8.2)**.

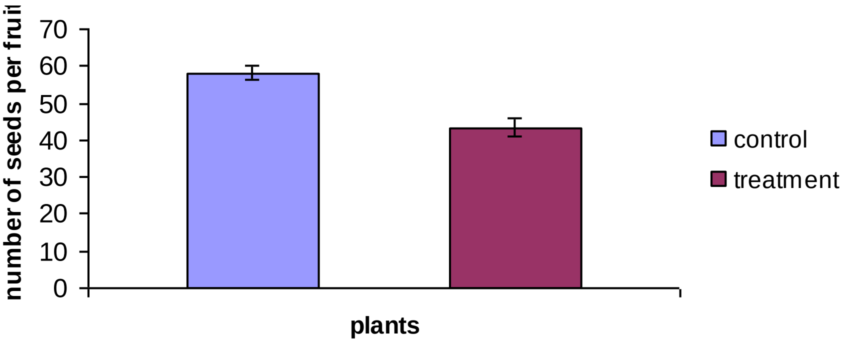


Figure 4.8.2 Mean seed set per fruit for *P. campanularia*. Treatment plants seed production per fruit was significantly lower than in the control plants <0.0001 (t-test). Control plants produced a mean seed number of 58.1 ± 1.98 per capsule (n=50), treatment plants had a mean seed number of 43.46 ±2.21 per capsule (n=50). Error bars show standard error.

4.8.3 Effect of corolla spot removal on seed crop

The total seed crop was 47% greater in control plants than in treatment plants. The mean number of seeds produced per plant was significantly lower in treatment plants (P=0.001, t-test) **(fig. 4.8.3)**.

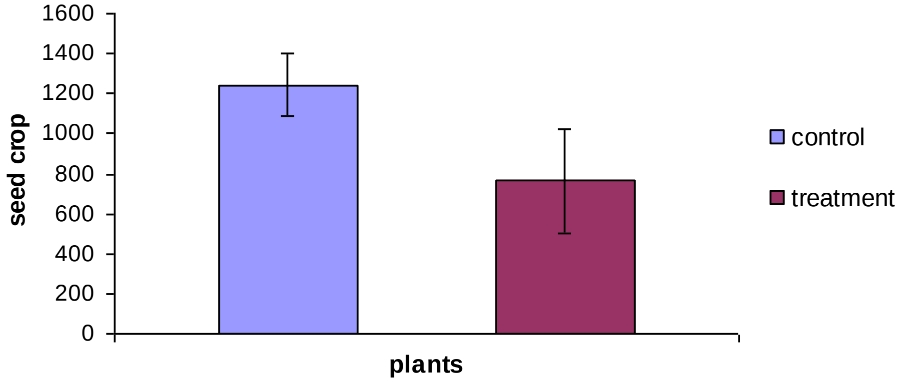


Figure 4.8.3 Seed crop. Treatment plants produced significantly less seeds per plant than controls P = 0.001 (t-test). The mean number of seed produced per plant by controls was 1,379 seeds ±156 Treatment plants produced 733seeds ± 256 (Error bars show 95% CI.)

4.8.4 Effect of corolla spot removal on seed mass

Seeds produced by the treatment plants had a significantly lower mass than seeds from control plants P < 0.0001 (t.test) **(fig. 4.8.4)**. Values were calculated from 50 fruit capsules harvested from ten different plants in each treatment.

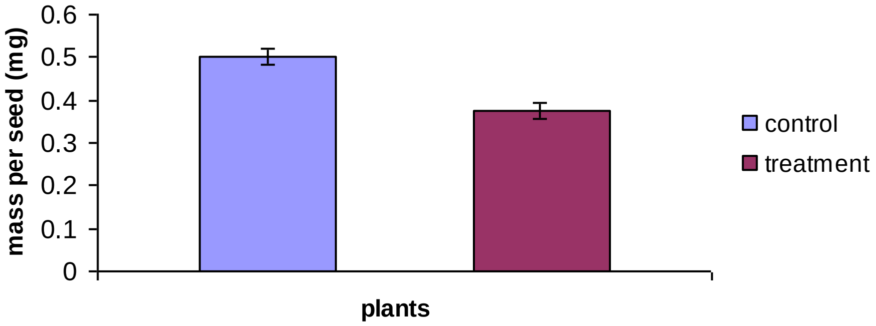


Figure 4.8.4 Seed mass for *P. campanularia*. Seed mass was significantly lower in treatment plants P < 0.0001 (t-test). Values shown were calculated from 50 fruit capsules harvested from ten different plants in each treatment. Control seeds had a mean mass of 0.501 mg ± 0.02 mg per seed; treatment plant seeds had a mass of 0.377 mg ± 0.018 mg per seed. Bars show standard error.

4.8.5 Artificial and natural pollination seed comparisons.

Seed number and seed mass obtained from the naturally pollinated (treatment and control) plants and the glass- house artificial pollinations were compared by ANOVA. The results revealed that the three means for the number of seeds differed by $P<0.0001$ and seed mass means differed at $P = 0.0002$.

Seed number from control plants and seed number from artificial pollinations did not differ but treatment plants produced significantly fewer seeds $P<0.05$ (calculated using LSD = 20. 68 corrected using Bonferroni's method) (**table 4.3**)

Table 4.3 Pairwise comparisons of means for seed number per fruit using LSD=20. 68 for the three treatments under different modes of pollination.

Type of pollination	**Natural pollination**		**Artificial pollination**
Treatments	control	treatment	hand pollinated
Mean Seed number (± 95%CI)	58.28 ± 7.47	33.28 ± 5.98	59.14 ± 4.97

Seed mass per seed from control plants and from artificial pollinations did not differ but treatment plants produced significantly fewer seeds $P<0.05$ (calculated using LSD = 0.058 (corrected for multiple comparison using Bonferroni's method) (**table 4.4**)

Table 4. 4 Pairwise comparisons of means for seed mass per seed (mg) using LSD = 0.058 for the three treatments under different modes of pollination.

Type of pollination	**Natural pollination**		**Artificial pollination**
Treatments	control	treatment	hand pollinated
Means seed mass (mg) (± 95%CI)	0.42 ± 0.058	0.27 ± 0.074	0.44 ± 0.056mg

4.9 Effects of staminode removal on the frequency of insect visits to *P.palustris* flowers.

The effect of staminode removal resulted in 29% fewer visits to treatment flowers than to control flowers**(see figure 4.9).** Treatment plants received significantly less visits than control plants P<0.0001 (Mann-Whitney U test).

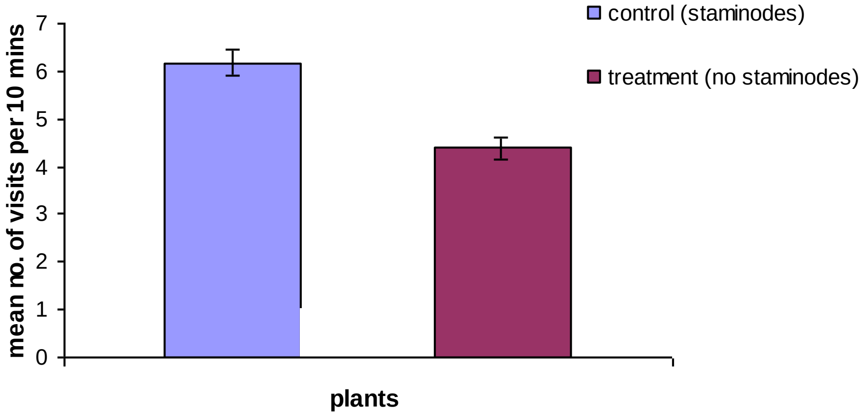


Figure 4.9 Visitor numbers to *P. palustris*. Treatment plants received significantly fewer visits than control plant P <0.0001 (Mann-Whitney U test). The mean number of visits received to treatment plants was 4.384 ± 0.39 visits per 10 minutes, control plants received 6.17 ± 0.41 visits per 10 minutes. Bars show standard error.

4.10 Variation in visitor spectrum

Visitors to *P. palustris* were mostly Diptera spp., these included various Syrphid and Asilidae species. Coleoptera, Nitidulidae, Ichneumonidae, Symphyta and Pompilidae were also recorded sitting in the flowers. The visitor spectrum varied in different weather conditions but was similar between control and treatments (**fig. 4.10**).

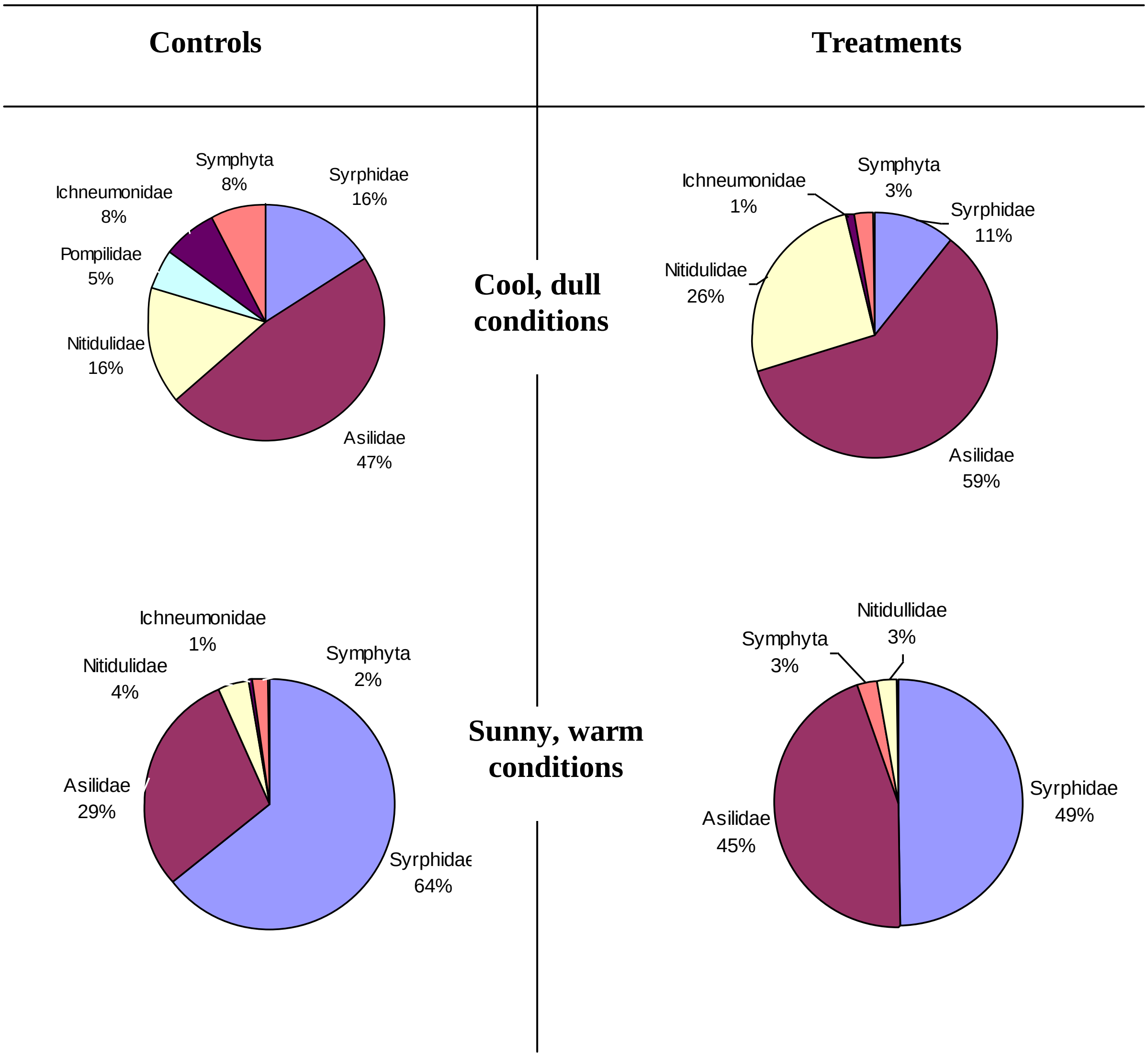


Figure 4.10 Visitor spectrum to control and treatment flowers under differing weather conditions. In cool, dull conditions the spectrum showed a wider variety in visitors in the controls than in the treatments. Asilidae accounted for a larger range of the spectrum under these conditions. Syrphids were the predominant visitors to control flowers, but overall the spectrum was similar indicating similar responses to by insect to the presence of staminodes.

4. 11 Variation in insect visitors under differing weather conditions

On cool dull days Syrphid and Asilidae visitors showed no preference for treatment or control plants, but all other visitors showed a significant preference for plants with staminodes, P=0.001(Mann Whitney U test) **(figure 4.11.1).** The situation was different on warm sunny days when Syrphids were the most frequent visitor and also showed a significant preference for control plants P = 0.007 (Mann Whitney U test). Asilidae and other visitor were lower under these conditions and preference for treatment or control plants was not significant **(figure 4.11.2).**

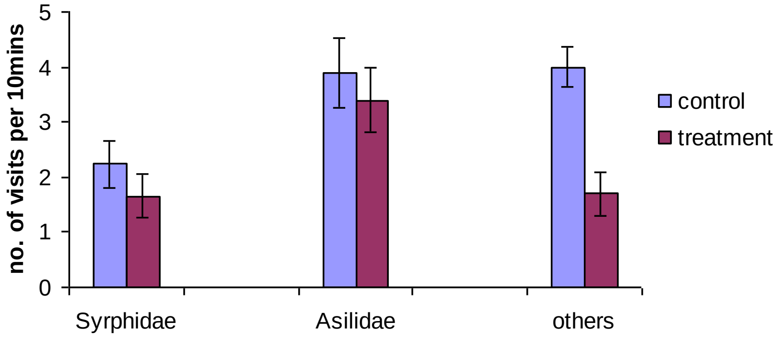


Table 4.11.1 Insect visitors on cool dull days. Syrphid and Asilidae visitors did not differ under these conditions, but all other visitors showed a preference for control plants. P=0.001 (Mann Whitney U test). Median number of visits to controls was: for Syrphids 2.24 ± 0.43, Asilidae 3.9 ± 0.63 and other insects 4.0 ± 0.37. Visits to treatments were: Syrphids 1.66 ± 0.39, Asilidae 3.4 ± 0.59 and others 1.7 ± 0.39. Bars show standard error.

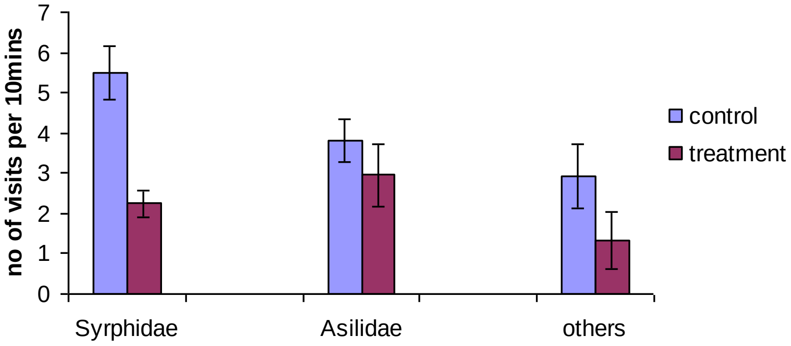


Figure 4.11.2 Insect visitors on sunny warm days. Syrphid visitors showed a significant preference for intact flowers under warm weather conditions P=0.0007 (Mann -Whitney U test). Asilidae visitors and other visitors showed no preference. Median number of visits to controls was: for Syrphids 5.5 ± 0.68, Asilidae 3.9 ± 0.53 and other insects 2.9 ± 0.78. Visits to treatments were: Syrphids 2.25 ± 0.68, Asilidae 2.96 ± 0.78 and others 1.33 ± 0.69. Bars show standard error.

4.12 Reproductive Success

4.12.1 Effects of staminode removal on fruit set

No significant difference was found in fruit set % between control and treatment plants (X^2_1 >0.05 Chi- squared test, with Yates correction). Fruit set was high in both control and treatment flowers, producing 86.6% and 73.3% fruit respectively. Fruit production in bagged flowers (in the absence of any visitors) was low with only one out of thirteen flowers setting fruit (**fig. 4.12**).

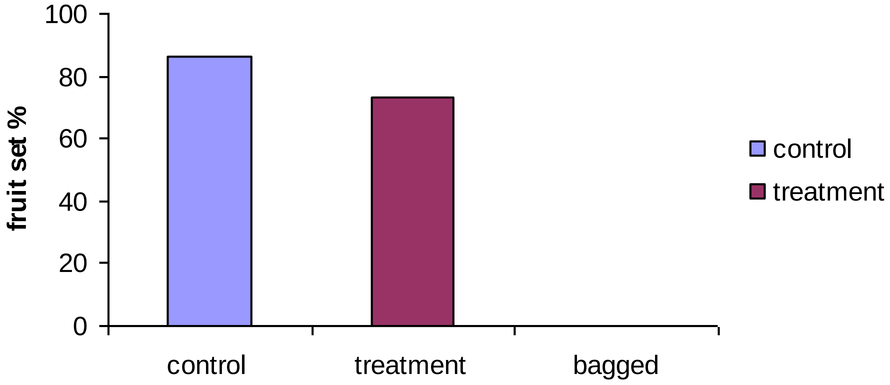


Figure 4.12 Fruit % for *P. palustris* . Fruit set did not differ between control and treatment plants (>0.05. Chi squared test). Controls produced 86.6% fruit, treatment plants produced 73.3% of fruit. Bagged flowers produced only one fruit.

4.12.2 Effect of staminode removal on fruit Size

Fruit capsules produced by treatment plants were smaller than those produced by the controls in both length and width, P= 0.02 and P= 0.0009 respectively (Mann Whitney U-test). **(fig 4.12.1 fig 4.12.2a and 4.12.2b)**. (Seed number in *P. palustris* was not counted but has been previously been shown to be highly correlated with seed size (Sandvik and Totland, 2003).

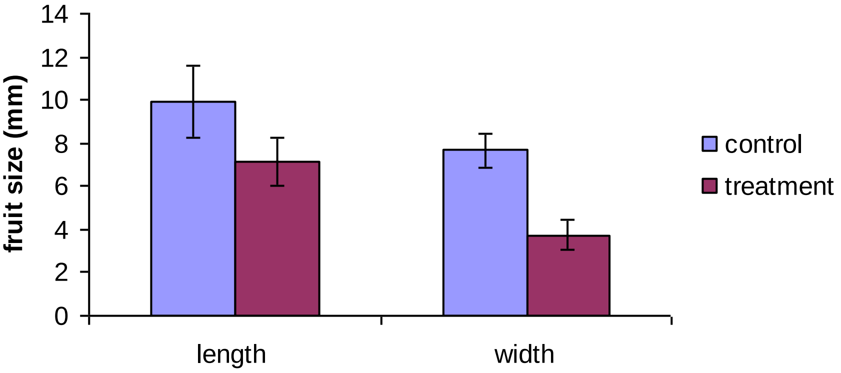


Figure 4.12.1 Fruit size. Fruits were significantly smaller in treatment plants P = 0.02 (for length) and P =0.0009 (for width) (Mann- Whitney U test). Median values for controls and treatment were fruit length was 9.9 mm and 7.15mm, median value for fruit width was 7.65mm and 3.75mm respectively. Bars show standard error.

Figure 4.12.2a Fruit capsules from control plants.

Figure 4.12.2b Fruit capsules from treatment plants showing smaller seed size.

4.12.3 Effects of staminode removal on seed mass

The mean seed mass produced per fruit was 60% lower in plants with staminodes removed P = 0.002 (t-test) bagged flower data was not analysed as only one fruit was produced which had a very low seed mass **(figure 4.12.3)**.

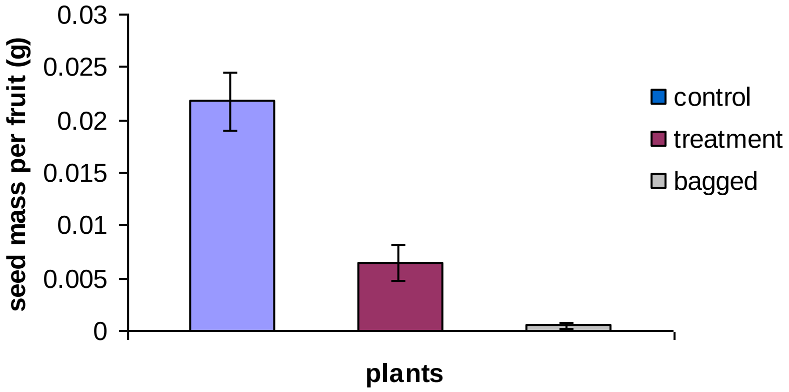


Figure 4.12.3 Total seed mass per fruit. Seed mass was significantly different between control treatment plants (P = 0.002, t-test). Plants with staminodes present (control) produced fruit capsules containing seeds with a total mass of 0.025 g ± 0.007 CI. Plants with staminodes removed (treatment) produced seeds with a mass of 0.01 g ± 0.003 CI. Bars show 95 % CI.

4.13 Effects of staminode removal on floral heating

When ambient temperatures were below 16ºC there was no evidence for floral heating in control or treatment flowers. When temperature rose above to 18ºC, flowers with staminodes experienced higher temperatures within the floral disc, P< 0.05 (ANOVA)

Flower temperature were 5.05 ºC higher than the surrounding air temperature in control plants and 2.49 ºC higher in treatment plants (at 20 ºC) **(fig. 4.13**).

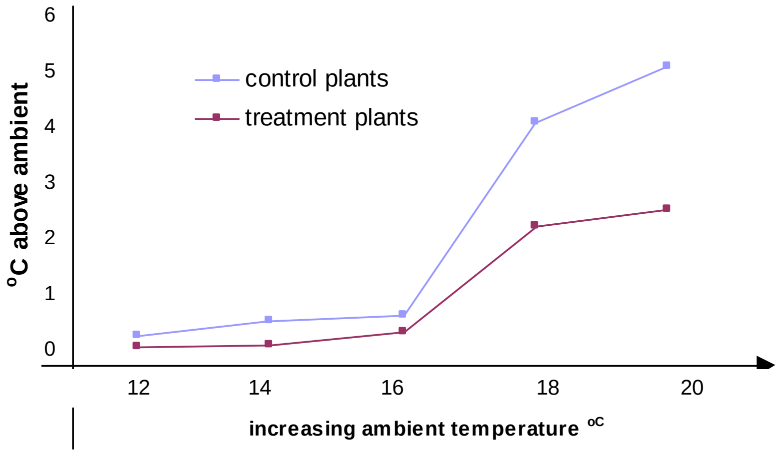


Figure 4.13 Floral heating in *P. palustris.* At lower ambient temperatures (below 16ºC) flower interior and ambient temperature did not differ but did differ at temperatures above 16 º C.

4.14 Temperature changes in male and female flowers

Temperatures were higher in male phase flowers than in flowers post pollination (or female phase), P <0.001 (ANOVA) (**figure 4.14**), which is consistent in the morphological shape change, with male flowers are cup shaped whereas post pollination flowers are flattened.

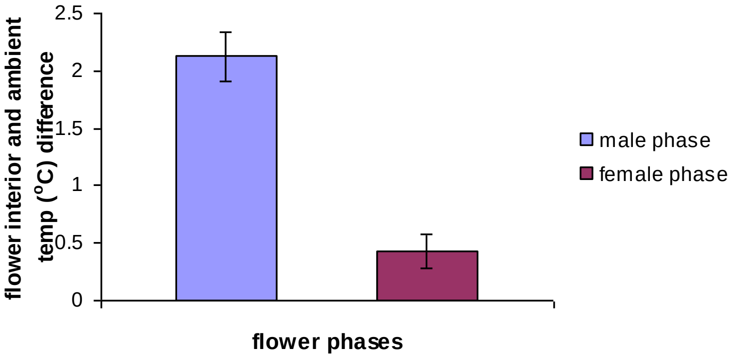


Figure 4.14 Flower phase temperature difference. In the male phase interior temperature is significantly higher than in post pollination flowers P< 0.001 (t-test). In male phase flowers temperatures were 2.13 ± 0.36°C above ambient temperatures. In female phase temperatures were 0.42 ± 0.31°C above ambient temperatures.

5. Discussion.

5.1 The impact of *Phacelia campanularia* petal markings on insect visitation.

Phacelia campanularia flowers received fewer visits by insects when the white corolla spots were disguised. The visitor spectrum was unaltered between control and treatment plants and this confirmed that UV reflectance patterns did not differ between the background petal colour and areas where paint was applied. These findings show that insects displayed a marked preference for flowers with corolla spots.

Throughout the study period the numbers of visitors to control and treatment plants was fairly constant, indicating that preferences for flowers with corolla markings occurred shortly after the plants were introduced into the garden environment. Learning experience did not appear to influence foraging behaviour despite the fact that food rewards were likely to be similar in all plants, suggesting that visual signals are important in decision making and flower choice. Both Bombus and Syrphid visitors displayed a positive response to the presence of corolla spots and this suggests that their perception of visuals signals was similar. Similarity in choice by both types of visitors is supported by the trichromatic visual system found in the majority of insects (Briscoe and Chittka, 2001).

Syrphids visited *P. campanularia* flowers more frequently than Bombus spp. and also exhibited a greater preference for flowers with corolla markings, occasionally they were also observed attempting to feed on the corolla spots. A possible explanation for such a preference is the pollen only feeding behaviour by Syphids on *P. campanularia* (probably because the nectaries are less accessible to syrphids). This may make Syphids more susceptible to visual stimulation by pollen mimicry in this flower. Bumblebees feed on both pollen and nectar and are often assumed to display preferences that are based on learned experiences, but within this study they exhibited only generalist and/or naïve behaviour by randomly feeding on several species of flowers. Flower constancy predicts that insects learn colours from randomly sampling the most rewarding species and then return to this colour (Heinrich, 1976). Flower constancy was not apparent in this study and this was probably due to the lack of available food sources within the coastal garden environment. According to Heinrich (1976), inner floral patterns are suggested as nectar guides once recognition has occurred. While this explanation for bee foraging behaviour has been shown to be true in bee training experiments the rules by which a naive or generalist insect evaluates flowers are most likely based on innate preferences and colour has been shown to be particularly important in initial flower recognition. Gumbert *et al*, (2000) showed that when honey bees and bumblebees were trained to specific colours and then exposed to completely different colours, they showed an initial preference for flowers within a the blue to violet wavelength. These choices corresponded to the same choices as those made by naïve bees. Most of the studies relating to innate colour preferences relate to single flower colours, but in reality many flowers exhibit more than one colour. Therefore if markings, such as the white spots of *P. campanularia*, match innate colour preferences, then they may be important when considering generalist pollinators and naïve insect behaviour within floral communities.

Osche (1983) argued that insect innate colour preferences arose from the early association with pollen colour. Lunau (1995) analysed pollen colour in 67 plant species and found that 75% had colours that are visible to humans in the yellow or white wavelength. Yellow to white coloured pollen was shown to be the most common colour in extant and current flower species. Lunau (2000) suggested that that pollen was the original attractant to insects prior to the development of larger floral structures and that many flowers enhance their visual signalling by mimicking pollen within their floral structures. Such markings are present in a large number of flowers across a wide variety of taxonomic groups and are often highly contrasting to the petal colours, particularly in blue flowers. This type of colouration is present in *P. campanularia,* which has corolla spots that closely match the colour of the pollen, and may therefore represent pollen colour mimicry. This was supported by the increased preference for flowers with spots in the study and is in agreement with the previous studies. Further support for pollen mimicry was presented in a recent study by Heuschan *et al*. (2005), the authors suggest that an innate neurosensory pollen filter mechanism exists in bumblebees, which is tuned to visual pollen signals. The authors found that bumblebees made more approaches to artificial flowers when they displayed highly contrasting central spot colouration. They explored Gumbert’s (2000) findings regarding the blue/violet preferences in naïve bees and showed that flowers were approached more often when the central spot colouration mimicked the pollen colour. They also found that pollen pigments and pollen mimicking spots were more consistently matched in colour than corolla colour pigmentation between individual flowers. These previous studies relate only to bees and no such studies have been performed with other insects, but the findings in *P. campanularia* suggest that a similar innate mechanism may be operating.

Given that pollen was the original attraction in flowers and insects share similar trichromatic visual systems it is not surprising that this kind of detection mechanism may exist across a broad spectrum of insects. Food signals may therefore be instrumental in naïve bee learning behaviour and to generalist pollinators and have evolutionary significance in plant reproductive biology.

This study shows that the presence of food signals needs to be considered within a plants their ecological context by taking into account the frequency of the flower species in question within that community and the choice of flowers available to generalist pollinators. Food signals appear to be particularly important in rare plants and in plants the have no food rewards. Gumbert (1999) showed in a study of plant communities that rare plants displayed a higher tendency to either converge in their colour signals, and that non rewarding species which are unable to train their pollinators may show high levels innate colour mimicry.

5.2 The impact of corolla spots on the reproductive success of *P. campanularia*

In *P. campanularia* the removal of the white corolla spots caused lower levels of reproductive success. The presence of spots appears to have an adaptive significance that may attract more visitors by enhancing floral rewards (as already discussed), and this is beneficial in cross-pollination.

The glass house studies showed that outcrossing was the predominant mode of reproduction in the breeding system of *P. campanularia.* Self-pollination experiments produced very low levels of fruit set which strongly suggests that these plants are highly dependent on pollinator movement. This apparent lack of selfing is unusual in an annual plant given that annuals are severely time limited, they must complete reproduction within one season and so are subjected to strong r-selection. Protandry may give some spatial separation that prevents selfing, but this does not prevent self-pollination within the same plant, unless the pollen is incompatible with the stigma. Consequently plants which produce large numbers of flowers may experience higher levels of selfing (Proctor *et al*, 1996). Thus many annual flowers are capable of self-pollination and this occurs as trade-off when cross-pollination fails within a given time frame (Snell and Aarssen, 2004). Selfing is thought to have evolved as a result of pollinator limitation caused by insufficient pollen donation or pollen arriving too late to allow fruit development within the season (Proctor *et al*. 1996)

Time limitation might be particularly important in *P. campanularia* within its native environment, which is the Mojave Desert, California. In these conditions it is more correctly described as an ephemeral, rather than an annual plant, the flowering period under such conditions is very short and competition for pollinators is high. Annuals account for around

15% of the worlds flora but in desert environments annuals and ephemerals represent a considerably greater proportion of the flora (Archibold, 1995). A selfing mechanism is common in ephemeral plants and it may be that within *P.campanularia's* native environment selfing does occur. No literature was available on this subject and further investigation is required to establish whether this is the case. The selfing experiments performed on *P. campanularia* showed that although selfing was rare, it did occur. It is therefore unlikely that genetic restraints prevent selfing, and it may only occur in response to certain environmental conditions. This type of response is reported to exist in some plants such as the annual Californian plant, *Clarkia xantiana,* which is out-crossing in larger populations but selfing in smaller isolated populations where pollinator limitation occurs (Runions and Gerber, 2000).

The lower rates of insect visitation resulted in insufficient pollen being delivered to *P. campanularia* stigmas; consequently, insufficient pollen was delivered to fertilise all the available ovules in the ovary. Pollen limitation may explain the lower fruit set in the treatment plants since a threshold quantity of pollen is sometimes required to stimulate fruit set (Wilcock and Neiland, 2002). Seed mass, as well as seed crop was also lower in treatment plants. In *P. campanularia* high seed crop is important within its native environment as seeds have to survive long periods of dormancy during which time they are subjected to severe predation (Beatley, 1967, Brown *et al*., 1979). Some animal populations, such as the Kangaroo rat, are reported to cycle in response to a reduction in an annual plants (Beatley, 1969). Several factors can lead to poor ovule development and pollen limitation or resource limitation are commonly cited as causes (Sutherland, 1987; Yang *et al.* 2005). However these two factors are not mutually exclusive and this study suggests that the resource limitation occurred as a result of pollen limitation. Resource limitation in *P. campanularia* treatment plants can be explained by the production of 33% more flowers. This excess did not occur in control plants because sufficient pollination occurred. Excess flower production is reported to occur in many self - incompatible plants which are reported to produce excess flower to fruit ratios as a bet–hedging mechanism which allows the plant to compensate for variation in pollinator rate (Burd, 1994)(Sutherland, 1986). Flowering usually ceases in response to pollination or after a given time period, but this did not occur in the treatment plants. These mechanistic changes ensure that resources are allocated to fruit development. In the absence of adequate fruit set flowering continued in *P. campanularia.* Anecdotal evidence of a similar physiological response is reported in the gardening practice of deadheading flowers. For many years gardeners have removed the flower heads annual plants to prevent fruit set and this facilitates continued flowering over a longer period (although despite an extensive search no scientific literature could be found relating to this practice). Extended flowering period may increases the number of flowers available to pollinators (and therefore pollen donation and available ovules) but this is a risky option in a time-limited annual. Even if cross-pollination does occur under these circumstances, it is likely that the plant will run out of time to complete fruit maturation. Thus resource limitation may explain the smaller seed mass in *P. campanularia*. The same resource limitation did not occur in open pollination plants and this was confirmed by glass house cross pollination's in which seed number and seed mass were shown to be similar to the open pollinated control plants.

Lack of a reproductive assurance from selfing in *P. campanularia* means that food signalling is all-important in this plant. The evolutionary significance of corolla spots is reflected by insect choice for plants with spots and this may reflect their innate colour preferences. Insect choice for flowers with spots directly affected reproductive success. When corolla spots were disguised plants produced lower seed crops than plants with spots present. High seed crops are important in desert plants, as many seeds can be lost to predation during long periods of dormancy. Therefore signals which mimic food may be evolutionary selective factors in the reproductive biology of certain plants.

5.3 The impact of staminodes on insect visitation to P*arnassia palustris* flowers

In *P. palustris* insect visitation rates to flowers with their staminodes removed were lower than visits to flowers with staminodes and this supports Sandvik and Totlands (2003) study. Both of these studies and also Kulger (1956) (cited in Proctor *et al* 1996) have shown that flies, and in particular Syrphids, have been observed to be initially attracted to, and attempt to forage on, the staminode tips before finding the true flower rewards. The food mimicry hypothesis is supported by these observations. In addition the colour of the staminode tips closely matches that of the nectar secreting bases, which is yellow when the flower is in the receptive phase (see *P. campanularia* discussion). Previous studies have not related the importance of staminodes to different insect visitors, but in the Gardenstown investigation the interaction between visitors was also examined within the context of the native environment. This revealed that although some generalist visitors are attracted by staminodes, other predatory visitors are not. These predators were identified as various Asilidae or robber flies (Diptera). These flies lie in wait for visitors and are present in *P. palustris* flowers irrespective of the presence of staminodes. They are the predominant visitors in cool dull weather when a wide variety of other insect visitors (Coeleoptera, Diptera spp. etc.) are also observed in the flowers.

In warm sunny conditions Syrphids are the predominant visitors and exhibit a strong preference for flowers with staminodes. Asilidae are opportunistic predators, as described by Werner and Pont (2003) and are known to predate a wide variety of insects, including bees, wasps, flies and moths. It is likely that they use *P. palustris* to lie in wait for flower visitors and may be dependent on insects that are attracted by staminodes. Asilidae are more abundant in sunny warm conditions as they like to sit in a sunny position and wait for prey (Chinery, 2005). Robber flies are known to catch their prey in mid air, stabbing and injecting them with saliva that contains neurotoxic and proteolytic enzymes. These enzymes paralyse and digest the proteins in the body. The robber fly will then returns to the same flower to and consumes the prey. It is possible that Asilidae may have a negative effect on cross-pollination if they are predators of Syphids (further observations are required to confirm this).

The study in Gardenstown indicated that a complex food web may exist which is important in *P. palustis* pollination. It is important to identify the role of visitors as often the *most common* visitors may not contribute to pollination, but, the same species within a different environment the opposite may be true (Fenster, 2004). Certain visitors may be detrimental to pollination success and consequently studies which ‘lump’ all visitors together may give misleading results (Memmott, 2004.)

5.4 The impact of staminodes on the reproductive success of *Parnassia palustris*

This study and others found, that when considering all insect visitors, that the presence of staminodes increased insect visitation rates. But Sandvik and Totland (2003) found that the reproductive success differed in populations at separate sites (Lista and Finse in Norway). They stated that the importance of staminodes varied depending on the elevation of the sites. The site at higher elevation suffered lower levels of fruit set as a result of resource limitation (poor rapidly changing weather conditions were also cited but not considered in their analysis). They also found that visitation rates were highly variable both during the season and between years. They did not consider any other factors (such as temperature, activities of different insect types or distribution). But concluded that at the elevated site insects facilitated pollination, whereas at sea level flowers were cross-pollinated. This difference can be attributed to the different types of insects, which, in Gardenstown, were shown to vary under different weather conditions (as mentioned previously). In Gardenstown bagging experiments to test for self-pollination showed that *P. palustris* is self-incompatible and therefore cross-pollination is the method of reproduction at this location. However under different environmental conditions pollinators may be limited, and the mode of pollination may change from cross to self-pollination. Different modes of pollination are reported at differing locations in a number of flowers, such as *Ophrys apifera* (Barth, 1985), and in some species this has resulted in sub-species of out-crossing and self pollinating plants (Runions and Gerber, 2000).

The number of fruit set within the Gardenstown population of *P. palustris* did not differ between control and treatment plants. This was because visitor numbers to plants were sufficient to initiate fruit set in all flowers. In addition these perennial plants are not under the same pressures as *P. campanularia* to complete their reproductive cycle within a short time frame or to produce the same large seed crops. Seed production and seed mass and size were all lower in plants when staminodes were absent. The most likely cause of lower seed set is that a lower amount of pollen and /or quality of pollen is imported. Control and treatment plants were competing for pollinators and those without staminodes received 27% fewer visits, therefore the amount of pollen (although sufficient to set fruit) may have been insufficient in terms of maximum seed set. Burd (1994) stated that, pollen limitation was the common cause of low seed set, his study surveyed of 258 species and found that 62% suffered low seed set from pollen limitation. This may be attributed too little pollen being delivered too late, or pollen from other plants being delivered by insect that are not pollinators. However, in P. *palustis,* staminodes may play an additional role in acting as a barrier between non-pollinatng insect visitors and the stigma, which prevents the delivery of self pollen to the stigma (Fenster, *et al*. 2004). Non- pollinating visitors may bring pollen from other species of plants or self pollen, and where a plant is self incompatible, pollen clogging may occur as their is only a limited amount of space on the stigma (Wilcock and Neiland, 2002). Pollen clogging was likely to have been a contributing factor to lower seed set because the absence of staminodes allowed more non-pollinating visitors to come into contact with the stigma. It was not possible to study the pollen load in this plant due to the large size of the stigmatic surface and attack by fungi in the field environment Therefore confirmation of pollen load and type as contributing factors could not be confirmed and further investigation is required.

Asilidae carry a large amount of *P. palustris* pollen, but do not move from flower to flower, but instead, sit in wait for prey within the flowers. Many other small visitors, such as the Coleopteran spp., carry only small amounts or no pollen. Syrphids are the most likely facilitators of cross-pollination as they carry a considerable amount of pollen and also move in rapid succession between flowers. They were also reported by Sandvik and Totland (2003) to be the most common visitor at the sites with the higher fruit and seed set. Although in Gardenstown, pollinator limitation did not occur, at some sites it does occur and self-pollination can be operational in different conditions. Sandvik and Totland, (2003) demonstrated that *P. palustris* reproduction differs with self and outcrossing populations at the two sites. The differences resulted in lower pollinator movement, in this case unfavourable weather conditions were likely to reduce Syrphid movement. At Gardenstown the importance of staminodes was demonstrated as an initial attractant to the primary pollinators and other insects, but may also have other roles in different environments.

Sandvik and Totland (2003) suggested that thermal heating was present in *P. Palustris* and that this might be attracting insects. However they failed to differentiate between thermal heating under different weather conditions and the differing roles of visitors. At Gardenstown, flower heating was only found to occur at temperatures above 16ºC, and it is at temperatures below 16ºC that the majority of insects are found within the flowers. However flowers in the male phase are warmer beneath the staminodes than flowers in the female phase or post pollination and this is consistent with the floral phenology examination which shows that a shape change occurs following pollination that discourages insects from sitting in the flowers.

It is therefore suggested that thermal heating may occur as a form of heliotropism as described in a previous study by Galen (2003). The author demonstrated that heliotropism promotes pollen quality and pollen germination in *Ranunculus adoneus,* which like *P. palustris,* is a disk shaped flower. The effect of warm sunny conditions appears to create a microenvironment within the flower disk; such heating is beneficial to pollen germination and tube growth. The presence of the staminodes in *P. palustris* appears to add to this effect in sunny conditions at temperatures above 16º C. Heliotropism may also be a contributing factor in the higher seed set at the sea level site in Sandvik and Totland (2003) study.

5.5 Summary

The results of these studies support the hypothesis that food mimicry signals are present in *Phacelia campanularia* and *Parnassia palustris* and that their presence had a positive impact on insect visitation and reproductive success. But that the roles of food signals may vary under different environmental conditions, and this may result in different modes of reproduction. The importance of false food signals should therefore be considered in their full ecological context

5.6 Suggestions for future studies

Although the findings in this study were in agreement with the hypotheses, certain additions would have been beneficial in adding further support to the findings. Because two separate plants were studied in different environments, this study was severely time limited by the flowering periods and the associated pollinator life cycle at two separate sites. Ideally a field pollination study should be carried out over more than one season to establish any variations within and between seasons.

In the case of *Phacelia campanularia* it would have been beneficial to test the food mimicry hypothesis further by including extra treatment groups. Two extra groups would have been beneficial in this study: i) the addition of extra white spots added to corolla petals and ii) removing the anthers to determine whether insects still approached the flowers in the absence of pollen.

In *Parnassia palustris*, only a small number of flowers were used in the study because it is a native plant that is relatively low in abundance in the area. The following treatments would be useful in any future investigations: i) to hand pollinate flowers with excess pollen in order to compare seed number and size in all groups. This would have allowed a more accurate assessment of whether or not pollen limitation or pollen clogging was the cause of lower seed set (in light of the fact that pollen load proved very difficult to count on this *P. palustris* stigmas). ii) Removal of anthers would have categorically confirmed cross-pollination as the only possible method of reproduction. A further breakdown of insect species identification would have been useful to determine the possible interactions within the floral communities as they may also influence plant reproduction. Longer observation time and video footage would be beneficial in assessing insect behaviour

Spectral colour reflectance of pollen and flower colours would also be beneficial to confirm whether they were in agreement with Heuschen, 2005, findings.

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