WHY DO FLOWERS IN NAMAQUALAND CLOSE?

Flower closure in relation to the environment and pollen sensitivity to moisture

---

AMREI VON HASE
Botany Honours
University of Cape Town

Supervisor:
Professor RICHARD COWLING

Key-words: Flower opening and closure, desert system, temperature cue, moisture, pollen viability and damage, flowering season.

ABSTRACT

Namaqualand, South Africa, is a desert system characterised by predictable winter rainfall and mild temperatures. Flowering coincides with the wet season which imposes constraints on pollination success. The wide-spread phenomenon of flower closure in the flora may represent an adaptation for protecting sensitive pollen from damage by moisture (dew, rain). The literature dealing with the subject is sparse and we addressed this gap by investigating patterns of flower closure in relation with environmental variables (potential cues). We also determined the effect of water on pollen in field and laboratory situations.

The findings are that air temperature closely reflects moisture levels and is the cue for diurnal patterns in flower closure. Of the abiotic factors tested, it best explains the biological variable of flower temperature which is closely correlated with flower closure. Variation among species in their response to ambient temperature is demonstrated by individual thresholds for flower opening and differing strengths of the relationship.

The detrimental effect of moisture on pollen viability emerges in four species (Mesembryanthemaceae, Asteraceae) where exposure to water caused significant pollen damage. Field experiments on two of these species confirm significant damage under natural conditions. Petal closure is the dominant protective mechanism in these plants and effectively prevents losses in reproductive potential caused by moisture. This has evolutionary significance as many Namaqualand species persist via annual recruitment. In two species (Asteraceae) that do not close their petals above inflorescences, pollen viability was retained despite placement in water. They may have a different protective strategy, such as a germination inhibitor, or their pollen could be insensitive to water.

INTRODUCTION

Deserts are characterised by the scarcity of water which becomes the primary factor controlling biological processes[16]. The focus of study in such systems naturally tends to revolve around the difficulties posed by a low and frequently irregular supply of water[16] and around the mechanisms that have evolved within the fauna and flora to overcome the constraints[16, 17]. There is rarely mention of the problems that may be associated with the presence of water. This rather paradoxical concept will form the core of the present investigation into flower closure in Namaqualand, north-western South Africa.

Namaqualand is an unusual desert system forming part of the winter-rainfall Succulent Karoo Biome[1,2,15,22]. As in other arid lands of the world, annual rainfall is low, with most of the region receiving less than 150 mm[1,15]. What sets Namaqualand apart, though, is its unique selective regime, characterised by high rainfall predictability and mild temperatures throughout the year [1,2,15,24]. In the coastal zone, another source of moisture comes in the form of fog. This climate underlies many exceptional features within the region's flora [1,2,15], including high species diversity, the predominance of leaf-succulent, drought-sensitive shrubs, winter growth phenology and massive floral displays in late winter and spring[1,15]. The biology of individual plant species further reflects the importance of a reliable moisture regime as germination and seedling establishment is frequently determined by the presence of water[10,15]. Most members of the Mesembryanthemaceae (one of the largest families in the area) also depend upon water for seed dispersal as their seeds are locked into capsules opening only in response to moisture[10]. In these instances, the influence of the selective regime on survival, growth and reproduction in most of the plants is thus fundamentally driven by the need for water.

Yet, the timing of the flowering season, which largely coincides with the

rainy period, indicates that water itself, and not only the lack thereof, also imposes constraints on some plants and biological processes. It is known from Mediterranean ecosystems, for example, that flowering in the wet season is problematic due to the adverse effect of precipitation and lower temperatures on pollination success [4,6]. Many species seem to have evolved adaptations in response to the difficulties associated with flowering in unfavourable conditions [4,6,7,14].

The same may be true for Namaqualand as the phenomenon of flower closure occurs in a diverse array of species of different taxonomic affiliations (e.g. Asteraceae, Mesembryanthemaceae). Little is known about the dynamics involved as the literature on this kind of flower behaviour is sparse. Few studies deal with the phenomenon in particular[25] although several anecdotal, descriptive and speculative accounts[14,19,25] refer to flower closure. It does not seem to be a common feature of other arid regions (pers. comm. P. Rundel) but mention of its occurrence in various parts of the world [14,18,19,25] confirm that it is not unique to Namaqualand.

Yet, the strikingly wide-spread nature of flower closure in the region suggests that it may be a common adaptive feature to protect floral reproductive parts from exposure to unfavourable conditions. Temperature can be eliminated as a reason for flower closure, since it does not reach low enough levels (sub-zero) to be physiologically damaging[1,15,26]. It may, however, be the cue for flower behaviour as it is usually associated with humidity levels in the air. The rationale for isolating moisture as the most likely driving factor for flower closure lies in observations on the behaviour pattern[14,18,25]. The flowers generally open for the day but close overnight when fog and heavy precipitation of dew due to lower temperatures commonly occur[1,2,10,15,26]. Flowers also seem to close in rainy or cool weather. Assuming that floral parts are indeed sensitive to wetting and have no protective features other than petals (e.g. germination inhibitors to keep pollen viable despite contact with water [7]), then flower

closure would represent a great advantage in a region where the chance of being wetted is often substantial.

Pollen emerges in the literature as the plant component most likely to be adversely affected by moisture. Several speculative accounts [14,19,25] infer pollen sensitivity to wetting from floral morphology (e.g. pendulous flower heads) and behaviour (flower closure, pollen presentation). More direct evidence is produced by investigations concerned with optimum storage conditions for pollen[13,23,24,27]. Even though these are not concerned with ecological aspects relevant in the field [27], they show that pollen of most species, excepting grasses, maintains its viability best when kept under conditions of low humidity.

Ecological considerations are introduced in studies on plants of the Mediterranean region[4,7]. Pollen damage due to contact with water was compared in a range of summer and winter-flowering species[4]. It is found that moisture has a far more detrimental effect on pollen in species flowering during the dry season[4]. The interpretation is that pollen grains of these species are less resistant to water, as they are not normally exposed to wet conditions. Minimal pollen damage in species used to rainy conditions (winter-flowering plants) demonstrates the greater resistance of their pollen grains to water. The authors suggest that pollen damage in the field is rare, since it is closely related to the likelihood of being wetted. Plants thus seem to invest in the protection of their pollen only if the need arises. This makes sense in an evolutionary context.

A similar reasoning underpins the results of work on two *Primula* species[7]. Evidence of a germination inhibitor is found in the species with ascending flowers, where floral parts are wetted in rain[7]. In the other species with pendent flowers, pollen is not usually exposed to rain drops. Upon wetting, it germinates[7] and its reproductive potential is lost to the plant. Thus different mechanisms exist for protecting pollen from losing viability under moist conditions.

The findings of these studies again establish a link between the Mediterranean and Namaqualand flora. Central to this link is the role of winter flowering, effects of moisture on reproductive output (pollen viability) and adaptations to increase pollination success under adverse conditions. In view of these aspects, the following will form the hypothesis under investigation:

The pattern of flower closure is linked to temperature and moisture levels. Temperature provides the cue for the behaviour, with flowers opening at high temperatures and closing under low temperatures. Under adverse weather conditions and at night, when temperature is low and moisture levels high, flowers close their petals around exposed sensitive pollen to protect it from moisture. The fundamental reason for flower closure is therefore detrimental effect of moisture, as rain and dew can cause pollen to be lost (washed away) or damaged. As flower closure provides the crucial protective mechanism, pollen is rarely exposed to moisture. Thus, damage is expected to be considerable when pollen does come into contact with water.

The approach of this investigation into flower closure follows these key questions:

1. What are the patterns of flower behaviour and what environmental factors are linked with these?
2. Which environmental factor is the cue determining opening and closing?
3. Can moisture be the driving force for flower closure?
   ~ Do moisture levels increase at night?
   ~ Does water damage pollen?

METHODS

Nomenclature:

This follows the Bolus Herbarium, University of Cape Town.

Site description:

The study was conducted at four sites within the coastal strip south of the Groen River mouth in Namaqualand, South Africa (figure 1, figure A appended). The vegetation in this area is classified as short and medium strandveld [1,15]. Asteraceae and Mesembryanthemaceae are the dominant plant families and there is a large component of succulent species [1,15]. The soils vary in depth, from very shallow nearest the coast to deeper further inland [1]. A shallow sandy A horizon and a red apedal B-horizon overlie the impenetrable subsoil, 'dorbank' [1]. The principal rainfall season is in winter, with peaks occurring between June and August[24]. Mean annual rainfall is approximately 140mm. The flowering season extends from July to September[1].

Site 1 (31°01'00"S 17°41'50"E) is located approximately 200 m from the sea (figure Aa). The dominant components of the vegetation are *Ruschia hutchinsonia, Othonna opima, Didelta carnosa* var. *tomentosa, Galenia fructicosa, R. fugitans* and patchily distributed *Delosperma* sp. Site 2 (31°01'30"S 17°42'10"E) lies 900 m from the coast on a gentle slope of about 10° (figure Ab). The vegetation is characterised by *Eriocephalus africanus, Zygophyllum morgsana, Ruschia fructicosa, Othonna cylindrica, G. fructicosa, Manochlamys albicans* and *R. subpaniculata*. Site 3 (31°01'30"S 17°43'20"E) is furthest inland, 2500m from the coast (figure Ac). *Z. morgsana, Eriocephalus africanus, O. cylindrica, Osteospermum oppositifolium, Ehrharta calycina* and *R. subpaniculata* are common here. Site 4 (30°51'00"S 17°33'30"E) is 400 m from the sea, on a slight incline of about 15° (figure Ad). Dominant features in the vegetation are *C. spongiosum, O. cylindrica, Othonna opima, O. oppositifolium, R. fugitans, Galenia fructicosa* and *Pteronia onobromoides*.

Data collection took place over a period of four days (11 - 14 July 1998) at sites 1 to 3 and over 3 days (4 - 6 August 1998) at site 4.

Study species:

The species forming part of the study are all endemic to Namaqualand and the West Coast of South Africa. Note that the term flowers is used to refer to the floral parts of all study plants, irrespective of the presence of true flowers or inflorescences (as in the composite flower heads of the Asteraceae). The term flower behaviour in the text refers to the opening and closing actions of flowers. The habit of the different study species, their height, flower size, presence of leaf succulence and appearance of closed flowers are presented in Table 1. Specimens of the taxonomically problematic species *Delosperma* sp., *Cephalophyllum* sp., *Oxalis eckloniana* and *Ruschia subpaniculata* are deposited in the Bolus Herbarium.

Patterns of flower opening and closure

At each site, several plant species out in flower were monitored from morning (flower opening) till late afternoon (flower closure). *Delosperma* sp. and *Didelta carnosa* var. *tomentosa* were included at site 1, *Oxalis eckloniana, Lampranthus hoorlianus* and *Ruschia subpaniculata* at site 2, *Othonna cylindrica, Osteospermum oppositifolium* and *Ruschia subpaniculata* were monitored at site 3 and *Cephalophyllum spongiosum* and *O. cylindrica* at site 4. At regular intervals throughout the day, counts were conducted of the status of flowers - open, half-open (intermediate) or closed for each species. Plants were randomly selected on walks through the field and used in counts. In the case of bushes, 3 flowers per bush were assessed, starting randomly at any side of the bush. The total number of flowers monitored was 50 of each species at site 1, 100 per species at site 2 (excepting *L. hoorlianus* which had a maximum of 35 flowers at any time) and 60 of each species at site 3 and 4.

Trends in environmental variables:

At site 2 (July) and site 4 (August) an Amtec thermo-hydrograph was set up in the shade to compile a record of continuous ambient temperatures. Relative humidity of the air at site 2 was regularly measured throughout the day with the use of a hand-held digital temperature/humidity meter (Lutron HT-3004).

Additional information on climatic variables was collected at site 4 in August:

Wind speed (Deuto anemometer) and total radiation (Skye instruments radiation sensor) were measured at 5 - 6 different times during the day and a continuous record of humidity (Amtec thermo-hydrograph) was taken over 3 days.

Flower temperature:

Flower temperature was determined in *Cephalophyllum spongiosum* (n=32) and *Othonna cylindrica* flowers (n=30). This involved aiming a portable infrared temperature measurement device (Raytek, Raynger 3i^{TM} series) at the flower centre until this came into focus and noting the temperature once the reading had stabilised.

Investigation of pollen:

In the morning of day 2 in July and day 3 in August, fresh pollen of *O. cylindrica, O. oppositifolium, L. hoorlianus, C. spongiosum, C.* sp. and *Arctotis merxmuelleri* was collected. At that stage, anthesis had clearly occurred and pollen was visible on the anthers. In each species, pollen was taken from several young, healthy-looking flowers where the petals were turgid and reproductive parts intact. The collection method of was to tap pollen grains lightly off the anthers into a vial with the use of a clean dissecting needle. The amount of pollen obtained for each species was divided approximately in half. One part was used in a hydration experiment while the remainder formed the control.

Control pollen: Immediately following collection, control pollen was mounted onto microscope slides in the field. This was done by picking pollen grains up in a cube of

fuchsin-stained glycerol jelly that was then gently heated with a lighter to melt onto the slide. Care was taken not to let the glycerol boil.

Hydrated pollen: In order to hydrate pollen grains, they were placed in dark vials with a few drops of distilled water and left for 36 hours at ambient temperature. After the wetting period drops of hydrated pollen in water were placed on microscope slides. Once most of the water had evaporated (approximately 3 hours), the hydrated pollen was mounted in the same way as the controls.

Pollen directly from flowers: An additional experiment was performed on pollen grains of *L. hoorlianus* and *C. spongiosum*. This was to explore the effect of moisture on pollen under natural conditions by manipulating flowers overnight. The petals of 4 flowers on a *L. hoorlianus* bush were tied back with elastics to prevent them from closing. This exposed the pollen to the surrounding environment. Four naturally closing flowers on the same bush represented the control. Similarly, in *C. spongiosum* 3 flowers on separate plants were manipulated to prevent closure. Three naturally closing flowers, one on each plant, were used as controls. Pollen of all flowers was harvested the following morning, at the time of flower opening, and transferred directly onto slides as previously described.

Pollen of the hydrated (naturally and in vials) and control treatments was viewed using a *Leica* compound microscope equipped with a camera. On each slide, 100 grains were counted around the edge and 100 in the middle of each slide to determine whether there was a qualitative difference in the state of pollen at these points on a slide. It has been reported that non-viable pollen tends to migrate to the edges of slides when using glycerol jelly to mount them[13]. Per species, a total of at least 400 grains was counted for each treatment. Pollen was classified as intact and damaged grains, with the latter category comprising burst, shrivelled, noticeably deformed grains and those where the intine was extruding from the apertures for more than half the length of the pollen grain or had ruptured. Burst pollen grains have also been observed in other studies [4,5].

Analysis of data:

The computer programmes Graph Pad Prism (Version 2.0), STATISTICA (Version 5) and Excel (Windows '95) were used in the analysis and presentation of data. Where necessary, a 'case-wise deletion of missing data' was performed in STATISTICA. This prevents bias in analyses as the corresponding observations for all variables are excluded when one variable has a missing data point [28].

Patterns of flower behaviour and environmental variables

Frequency data of flower counts was converted to percentages. Flower closure is characterised by the variable ' % flowers closed '. This category was used in analyses as no ambiguity is associated with it. Line graphs depicting trends in flower closure, ambient temperature and humidity during the study periods were generated. These serve to illustrate patterns in the data over time and required no further statistical analysis.

Relationships of flower closure in individual species and ambient temperature

Scatterplots of flower closure versus ambient temperature were created for *O. oppositifolium, O. cylindrica, C. spongiosum, D. carnosa* var. *tomentosa, Delosperma* sp., *R. subpaniculata, O. eckloniana* and *L. hoorlianus*. Non-linear regression methods were used to characterise the resulting relationships for each species. The assumptions of non-linear regressions are that the error term (residual) is a normally distributed random variable with a mean = 0 and equal variance for all values of x [28]. The dependent variable, being a function of the error term, must also have a normal distribution. Normality was assessed and accepted for all dependent variables by the routinely used Kolmogorov-Smirnoff (K-S) test. Even though the more rigorous Shapiro-Wilk's W test [29] rejected normality in the same instances, Monte Carlo studies suggest that it is not absolutely crucial to meet the normality assumptions

when the sample size is not very large [28]. This justifies the regression procedure subsequently followed.

As the aim was to compare the relationships of different species with temperature, the regression procedure was standardised and a common curve fitted for all species. The logic underpinning this approach is that the data sets differ only with respect to values of the dependent variable (% flowers closed) and the number of observations (n). As they are fitted to the same equation, Any variability in the shape of the curves generated will reflect differences in individual relationships. A range of non-linear equations (including all the common ones offered by Graph Pad Prism) was experimentally fitted to the data of each species to determine the most appropriate curve for the whole set of species. The Boltzmann sigmoid equation (Y = Bottom + (Top - Bottom)/(1 + exp ((V50 - X)/slope) was found to yield the highest correlation coefficients overall and produced the most appropriate curves. Thus it was used as a standard regression curve. The parameters 'Top' and 'Bottom' refer to the upper and lower limits of each curve as it levels out into a plateau. 'Slope' defines the slope of the entire regression line in each case and V50 is the x-value at which the dependent variable is at 50%. V50 will be denoted by $T_{50\%}$ (temperature at which 50% flowers are open). Note that the coefficient of determination (r^2) represents the proportion of the total variation explained by the fitted regression. It is therefore a measure of the strength of a relationship and of the 'goodness of fit' [28].

Relationship between flower temperature and flower closure

The strength of correlation between flower temperature and closure in *C. spongiosum* and *O. cylindrica* was determined using the non-parametric Spearman Rank Order test. Normality is not a requirement for this test [29]. These relationships were modelled using the Boltzmann sigmoid curve as above.

Relationship between flower temperature and environmental variables

In pursuing the search for a cue for flower closure, the potential effect of various environmental variables, other than temperature, needs to be taken into account. Flower temperature emerged as an excellent indicator of flower closure in the previous analysis. Any environmental factor determining flower temperature can therefore also be identified as the most likely cue for flower opening or closure. To examine this, multiple stepwise regressions were carried out using flower temperature of *C. spongiosum* and *O. cylindrica* as dependent variables. The assumptions of multiple regressions are the same as those specified above for non-linear regressions [28]. The data were tested using the K-S test for normality and fulfil the conditions. For both species, *C. spongiosum* and *O. cylindrica,* two separate stepwise analyses were run. The first included the independent variables ambient temperature (°C), wind (m/s), radiation (Q. Ir. *10 μmol/m^2/s) and humidity (% data, arcsin transformed). Wind was excluded from the second run. The final F-ratio was set to 1 (F to enter the equation) and 0.00 (F to remove from the equation). This prevents all variables from being forced into the equation and specifies that only the most important explanatory variables are taken into account during the analysis.

Dewpoint temperature

Dew point temperature (Td) at 100% relative humidity (R.H.) was calculated for 4 nights in August (3 - 6/8/1998). The equations

Td = (BC) / (A - C) with A = 7.5; B = 237.3; C = log(e/6.1078) and

e_s = 6.112 * 10^ ((17.67 - T) / (T + 243.5)) were used.

At 100% humidity, vapour pressure (e) = saturation vapour pressure (e_s).

Pollen analyses of control and hydration treatments

The differences in frequencies of damaged and intact pollen between the slide middle and edge were negligible. Thus data for middle and edge were combined and then

analysed. The relevant null hypothesis (H_o) is that there is no significant difference between the two treatments with respect to the frequencies of intact and damaged pollen grains. The alternative hypothesis states that the the hydrated treatment has significantly more damaged and fewer intact grains in comparison with the control. In each species pollen counts per category (e.g. control intact, hydrated damaged) were pooled and cumulative frequencies analysed. In the 'natural experiment' for *C. spongiosum* and *O. cylindrica* pollen of different flowers was kept separate and the means and variances were calculated for each category. Subsequently, these frequencies were combined for further analysis. Two by two (2 * 2) contingency tables were employed to compare the number of intact and damaged grains in the control and hydrated pollen for each species. In generating chi-squared values, Yates' correction term for continuity was taken into account. It is a more conservative estimate than the Pearson chi-squared statistic and must be employed when the degrees of freedom equal 1. This is applies even when high frequencies (n) are used in the analysis [28].
For each species the frequencies of damaged and intact pollen grains in control and hydration treatments were converted to percentages and graphed as bar graphs. Displaying the results as percentages allows comparison of different species.

RESULTS

PATTERNS and CUE

Description of patterns in flower behaviour in relation to air temperature:

Figure 3

Only four of the eight species monitored are shown in figure 3, as they give a good overview of the general patterns encountered. As ambient temperature rises, flowers begin to open and when it drops, flowers close. At peak temperatures the flowers of all species open (figure 3, day 1-3), whereas at low temperatures some species keep their flowers closed. The diurnal pattern in flower behaviour is most clearly seen on the first two days where ambient temperature follows a regular and predictable course. Flowers open fairly rapidly in the morning, display a long open period and close in the afternoon for the night. This generalised trend applies to all species (including those not displayed on the graph, e.g. *O. cylindrica, L. hoorlianus, C. spongiosum* and *Cephalophyllum* sp.) and is reflected in the similar shape of their curves. Slight variation exists in the exact timing of opening and closing. *Delosperma* sp. *and O. eckloniana*, for example, open later than most other species, while *O. oppositifolium* starts opening its flowers early (figure 3).

Deviations from the generalised trend become more pronounced on the last two days where temperature displays a less regular curve and is notably lower (<19 °C on day 4, figure 3). Under these conditions, flower opening in *Delosperma* sp. and *O. eckloniana* is delayed (day 3) or does not take place (day 4). A slightly different pattern is seen in *O. oppositifolium* and *R. subpaniculata* (figure 3), demonstrating that variation in flower behaviour does exist between species. These two species do not deviate from the generalised trend of flower opening on day 3. However, on day 4 only a portion of their flowers open for a short time in the afternoon (figure 3).

The patterns in figure 3 indicate that flower closure is linked to temperature but that differences in the response exist between species. As the data are purely descriptive a closer, more rigorous analysis further explores the relationship of individual species and ambient temperature.

Relationship of each species with ambient temperature:

Figure 4:

A good fit of the Boltzmann sigmoid curve is obtained for *R. subpaniculata* and a reasonable fit for *O. cylindrica, O. oppositifolium, , O. eckloniana* and *L. hoorlianus* (figure 4). In these species, the model chosen to represent the relationship is appropriate. The coefficient of determination (r^2) is highest in *R. subpaniculata,* followed by *O. oppositifolium* and *O. cylindrica* (figure 4). In these instances, the fitted regression explains 89.67%, 60.78% and 69.63%, respectively, of the variation in flower closure. These values point towards a relatively strong relationship with temperature. In *O. eckloniana* and *L. hoorlianus* the variation explained is below 60% due to more pronounced scatter of the data points.

Weaker relationships between flower closure and ambient temperature are displayed by *C. spongiosum, Delosperma* sp. and *D. carnosa* var. *tomentosa.* The low coefficients of determination indicate a poorer fit of the Boltzmann curve, which only explains approximately 50% of the variability in flower closure in these species (figure 4). It is likely that a different equation would better describe the relationship of these species with temperature. Yet, the intention was to compare the whole set of species on equal principles.

Even though the curves describing the relationship are not identical in all species, a common shape can be identified. Exceptions are *L. hoorlianus* and *Didelta carnosa* var. *tomentosa* which show the greatest deviation from the general shape, since no upper plateau is defined by their curves (figure 4). Each species examined has a particular

threshold temperature at which 50% of its flowers are opened. These thresholds range between 17.5 and 21.6°C (figure 4). Opening in some species occurs over a wider temperature range (e.g. *O. cylindrica*) than in others (e.g. *R. subpaniculata*). Thus, variations in response lie primarily in the timing and speed of flower opening.

Relationship between flower and ambient temperature

Figure 5:

The two temperature variables show a close link (figure 5) with the flowers of both *C. spongiosum* and *O. cylindrica* tracking ambient conditions. Their temperatures, therefore, appear to depend largely on those of their surroundings. Measurements of individual flowers of O. cylindrica and especially *C. spongiosum* in the field, however, frequently indicated flower temperatures that were notably higher than the surrounding air. In some instances a 10°C difference in temperature was found. These observations are only hinted at in the data for day 1 (figure 5) where the curves for mean flower temperature in the middle of the day are slightly higher than air temperatures. Flower closure, depicted on the graphs below (figure 5), is again inversely related to temperature, including both ambient and flower temperature in this case.

Figure 6

The correlation between flower temperature and closure (figure 6) is highly significant ($p < 0.001$) and confirms a strong link between these variables. This suggests that flower temperature is a good indicator of flower closure and opening. The regression curves show a relatively close fit in both species. The coefficient of determination (r^2) is significantly raised in *C. spongiosum* when compared with the r^2 value for its relationship with ambient temperature.

Further analysis will show whether flower closure is explained by ambient temperature, through establishing a link between ambient and flower temperature.

The influence of several environmental variables:

Explanatory variables for flower temperature and, indirectly, flower closure

Table 2.

The multiple regression performed for *C. spongiosum* and *O. cylindrica* highlights ambient temperature as the only highly significant explanatory variable for flower temperature. The placement of wind as a second, if rather insignificant (table 1), explanatory variable ($p < 0.5$) in the case of *C. spongiosum* is a surprising result. Examination of the raw data reveals that wind increases during the day (figure C, appended) and is thus negatively related with flower temperature. Radiation was found to be the second explanatory variable for *O. cylindrica* but only accounts for 1.32 % of the variance in flower temperature (table 2). It is of no great significance ($p < 0.5$). Humidity does not feature as an explanatory factor in the results of the multiple regression for either species.

REASONS for FLOWER CLOSURE

Moisture at night and dew formation:

Figure 7

During the August study period, water droplets were observed on the vegetative and floral structures of the study plants every morning (figure 2). Heavy rain only fell on the first night (3/8/98) and left large amounts of moisture on the plants. On all four nights, however, the level of relative humidity reached 100% (figure 7) and remained at that point for 12 hours on average (S.D. = 2.5 hours, n=4). The corresponding values for dew point temperature (Td) at 100% relative humidity varied from 1.5 to 4 °C, depending on the air temperature (figure 7). Humidity is closely linked to temperature (figure 7). Flower temperature was not measured at night, but early in the morning it was between 3.5 and 4.5 °C lower than ambient temperature (figure 5a, b). Dew

formation at night is therefore highly likely which explains the source of moisture seen on petals in the morning.

Pollen grain response to wetting:

Figure 8

The number of intact and damaged pollen grains in hydration versus control treatments yielded highly significant results ($p < 0.001$) for all species except *O. cylindrica* and *O. oppositifolium* (figure 8). Thus, the null hypothesis (Ho) is only accepted in those two cases where pollen damage after hydration is minimal. In both species, pollen in control and hydration treatments comprised a large proportion of grains where the intine extruded slightly at the apertures. The exine and intine were both intact and the intine only emerged far enough to form very small bulges at the apertures. Such pollen was classified as intact, since it is likely to have retained its viability and germinative capacity[11]. As both the control and hydration treatment revealed the same phenomenon, it may be regarded as a consequence of the mounting methods used. It therefore has no impact on the interpretation of the results.

For all other species (3 Mesembryanthemaceae, 1 Asteraceae) the alternate hypothesis is valid, stating that there are significantly more damaged grains in hydrated pollen than in the controls ($p < 0.001$). The greatest damage occurred in hydrated pollen of *L. hoorlianus, A. merxmuelleri* and *C spongiosum,* where chi-squared values are very high (figure 8). These species all close their petals over the central disc overnight, keeping reproductive parts from being exposed. Both experiments ('artificial' and 'natural') conducted for *L. hoorlianus* and *C. spongiosum* show significant damage in 'hydrated' pollen although the proportion of damaged grains is higher in the artificial hydration treatment (figure 8).

DISCUSSION

PATTERNS AND CUE:

Air temperature has an important influence on pollination and reproductive success as it affects flower development and opening of buds, nectar secretion, anther dehiscence, activities of flower-visiting insects and seed development[11]. In Namaqualand, temperature also influences the presence and diversity of drought-intolerant succulent plants that require mild night-time temperatures during the growing season[1].

In this study, temperature is singled out as the primary environmental cue for flower opening and closing. Evidence of this is first given in the descriptive part of the investigation, where a close link between flower behaviour and the trend in temperature can be traced (figure 3). Ambient temperature strongly seems to determine whether and when flowers of different species open and close. Thus, a regular temperature curve (figure 3, day 1 and 2) generates a correspondingly regular and generalised pattern of flower behaviour. This situation, with different species displaying similar responses, appears highly predictable as long as ambient temperatures reach relatively high values (above 20 °C, figure 3).

Differences in the precise timing of flower opening and closure indicate a degree of individuality in the response and may be linked to different temperature thresholds governing the behaviour. This is supported by observations that an irregular temperature curve and fairly low temperatures (below 20°C) disrupts the generalised pattern of behaviour (figure 3, day 3 and 4). Deviations of individual species become more apparent under these conditions. Such variation is probably an exaggeration of the differences observed in the first two days and again indicates that different temperature thresholds may limit the response of individual species.

These speculations on the role of temperature are based on purely descriptive data (figure 3). The illustrated trends suggest, but by no means confirm, a relationship between temperature and flower closure. A link could simply exist due to a common, though independent, diurnal pattern in these variables. In the light of further, more rigorous analyses this seems unlikely.

Relationship of individual species with air temperature

An exploration of the relationship between individual species and ambient temperature (figure 4) supports suggestions made with reference to patterns alone (figure 3). Variability in the shape of the curves shows that differences in the relationships exist. Some of the variation, especially among species where a good fit of the regression is achieved, can be accounted for by differences in temperature thresholds at which flowers open and close. This also applies to the weaker relationships, but to a lesser extent, as here, the validity of the model or of the relationship must be questioned. In these cases, it is likely that factors (other than differences in the timing of opening and closing alone) contribute to variation in the response.

The temperature threshold where 50% of flowers are open ($T_{50\%}$) differs for each species, although the range is fairly narrow (figure 4). This explains why flowers of different species do not open at the same time during the day (figure 3, day 1 - 3). On occasions where the flowers of particular species do not open at all (figure 3, day 4), the threshold for opening is not reached. *O. eckloniana* provides a good example. Its flowers generally start opening at temperatures above 20°C (figure 3) and remain closed on day 4 (figure 3) due to its high temperature threshold (figure 4). Other species have lower thresholds, such as *R. subpaniculata* which opens earlier in the day than *O. eckloniana* (figure 3) when it is still cooler.

The reasons underlying variation in thresholds and differences in the response to temperature (as reflected by strong and weak relationships, figure 4) are difficult to ascertain. Flower size (table 1) can be ruled out as a primary factor influencing the

timing of opening and closing. *Delosperma* sp and *R. subpaniculata*, for example, are the same size (table 1) but differ noticeably in their response and 50% threshold (20.6 and 17. 7 °C, respectively -figure 4). *O. cylindrica*, *O. oppositifolium* and *L. hoorlianus* have larger flowers (figure 1) but their 50% thresholds fall in between that of the small-flowered species (figure 3). Thus, no link exists between flower size and response to temperature.

A strong possibility for differences between species is their distribution relative to the site of temperature measurement (site 2, figure A). *Delosperma* sp and *D. carnosa* var. *tomentosa* were monitored close to the sea, where cooler sea breezes are common. These species are likely to have experienced a slightly different temperature regime, that is not picked up in measurements at site 2. Correspondence between flower data and temperatures (figure 3 and 4) is not therefore not complete. Pronounced scatter in the graphs of these two species occurs at the upper plateau (100% closed, figure 4). The flowers are thus still closed at fairly high temperatures. Yet, at those times, conditions at site 1 were probably cooler than at site 2.

Another reason for variation in the response of individual species to temperature may be that some flowers are 'messy closers'. Their response is not clear cut or consistent and they appear less sensitive to air temperatures. The link between flower closure and temperature is tenuous. A weak regression thus reflects the real situation. This applies to flowers of *D. carnosa* var. *tomentosa* which did not seem to follow a definite pattern in the field. Overall scatter in the data (figure 4) of this species bears proof thereof.

In species, where the fit of the regression was less tight (figure 4, *L. hoorlianus*, *D. carnosa* var. *tomentosa* and *Delosperma* sp.) three interpretations regarding the relationship with temperature are possible: Firstly, the regression model may be inappropriate and the relationship with temperature may best be described by a different equation. Secondly, there may only be a very weak relationship with temperature in these species. Thirdly, the data may not span a wide enough

temperature range (e.g. in *C. spongiosum*, where n = 16, figure 4) to reflect the relation with temperature adequately.

It is difficult to weigh these interpretations up against each other without pursuing each possibility in practice. Gathering more data over a wide temperature range would clarify whether the last suggestion is valid. More data points may allow more precise modelling of relationships. Alternatively, the fit of the regression curves could be improved using a different equation for each species. Such an approach would almost certainly yield equations that better describe the relationship with temperature for individual species. Yet, it would make comparison tricky or impossible and is not in line with the aims of the analysis, as it was originally defined. Thus, at present, it can be said that relationships with temperature differ among species and that one must speculate on reasons for such variation, as outlined above.

The link between flower closure, flower temperature and environmental factors

The significance of ambient temperature as a cue for flower behaviour is consolidated in the final analysis. This draws a biological variable - flower temperature with a direct influence on other processes in the plant - and other environmental factors into consideration (table 2). This step is essential, as previous analyses indicate a relationship between flower closure and ambient temperature but causality is difficult to establish. By linking flower closure to flower temperature and this in turn to environmental factors, their potential as a cue can be interpreted more reliably. In this way it can be established whether processes within the plant are independent from or influenced by external factors. Such an approach makes biological sense and therefore strengthens arguments based on correlative associations [28].

Flower closure is tightly correlated with flower temperature (r_s, figure 6) in both *O. cylindrica* and *C. spongiosum*. The fit of the sigmoid equation (figure 6, r^2) indicates that flower temperature is a good predictor for flower behaviour. The graph for *C. spongiosum* (figure 6) does show, however, that this equation is not entirely appropriate as a predictive tool for the data set, as the minimum value (lower plateau) occurs at a negative value (figure 6, and see table A, appended). This is not possible as the dependent variable is in the form of percentages and cannot reach below zero. In both species, these regressions yield a closer fit (higher r2) than the relation with ambient temperature (figure 4), indicating that flower temperature has a more direct effect on closure. This relationship is of importance, provided that flower temperature is involved in the opening and closing mechanism, perhaps by serving as a means for plants to perceive external conditions. It would thereby mediate flower response by acting as an indicator of environmental conditions.

From descriptive patterns (figure 5) the course of flower temperature seems to depend on the environment, most notably air temperature. This is expected unless flowers regulate their own temperatures. Interestingly, this does happen in some other plants even though they are not endothermic [31]. The advantages coupled with warmer flower temperatures may be pollinator attraction and enhanced growth and development in flowers [31]. Not much evidence of such a phenomenon is displayed in results of this study (figure 5 a, b) although observations during the warmest part of the day (figure 5) suggest that some the flowers (e.g. of *C. spongiosum*) possess the ability to heat up above ambient temperatures.

Yet, the overarching influence of ambient on flower temperature is satisfactorily established in the multiple regression analysis (table 3). This shows that flower temperature is not independent of ambient conditions. Air temperature emerges as the best explanatory variable of flower temperature, while other environmental variables seem to have a negligible influence (table 2). The interpretation of this

outcome is two-fold. Firstly, ambient temperature has the greatest influence on flower temperature (table 2). This is not surprising, as both are temperature variables. Secondly, though, it must be noted that environmental factors such as humidity, radiation and wind are in some way linked to temperature. Part of their explanatory power is incorporated into that of temperature [27,28]. Their role must therefore also be assessed separately.

Wind can certainly be eliminated as a cue, since an increase in wind (figure C, appended) is not conducive to flower opening, but rather has the opposite effect. Owing to this, and to its extreme variability, it cannot serve as a reliable cue.
Trends in radiation and temperature fit in with the diurnal pattern of flower opening and closing. The two variables have a fundamental link, as temperature often depends on radiation. In berg-wind conditions, however, where warm continental air is blown seawards, temperatures are high irrespective of radiation [20]. Under these conditions, flowers follow their regular course of opening and closing, even when it is overcast (personal observations in July, when radiation was not measured). Thus, direct effects of radiation on this behaviour seem minimal.
Humidity varies with temperature (figure 7) and pressure [20]. It does not override the effects of temperature [20], which is the ultimate determining factor.

A crucial question must be addressed before ambient temperature can finally be pronounced the primary cue for flower behaviour. Can a mechanism be envisaged by which temperature acts on flowers to bring about petal movement? Although no work has been done on Namaqualand plants in this regard, the following scenario is proposed. Temperature has a strong influence on the water status of plants as it controls the vapour pressure deficit in the air[20]. This is defined as the difference between the actual amount of water in the air and the saturation level at which evaporation equals condensation[20]. It is a measure of the dryness of air and a function of temperature alone[20]. As ambient temperatures rise in the day, vapour pressure

deficits increase since warm air can hold more water vapour than cooler air. This leads to higher evaporative demands, so plants transpire more and cells lose water. The resulting turgor changes provide the most likely mechanism, at the plant level, for the opening and closing of flowers. Alterations in turgor would need to be very controlled in the flower heads and take place in special cells that lose and gain water more easily than surrounding cells. The reason is, that most of the succulent study plants (excepting *O. cylindrica* and *O. oppositifolium*) flexible in their use of CAM (Crassulean Acid Metaboloism) and C3-photosynthesis [1, 13]. This enables them to close their stomata during the day which minimises transpirational water loss [13]. The special petal-moving cells should be positioned at crucial points for the directed opening and closing of petals, presumably at the base of the petals. Their response would be cued to external temperature and vapour pressure deficits, as perceived via floral temperature. In the morning, the petal-moving cells would lose water and turgor and become more flaccid. This causes petals to open. The reverse would occur in the afternoon, when temperature drops and the air becomes less dry (figure 7). Turgor is regained and petals are forced together. An investigation into the microstructure of flower heads would be necessary to verify the presence of cells that are responsible for a similar mechanism. The presence of a turgor-based system for flower opening and closure is conceivable, as it resembles those shown in other plants capable of movement, such as *Mimosa pudica* or *Dionaea muscipula* [9, 10], although these respond to different cues.

Ambient temperature is, thus, established as the dominant cue, acting on flower opening and closure. It determines humidity and closely reflects changes in moisture levels (figure 7). Thus, its diurnal pattern is a reliable cue for plants needing to respond rapidly (figure 3) to critical variations in moisture in their surroundings. Temperature is perceived by plants in a precise way (figure 5, a and b), so they warm up and cool down roughly in accordance with their environment. These points are in

keeping with the hypothesis originally formulated, and lead on to the second part of the study which examines the role of moisture and its effect on flowers and pollen.

THE REASON FOR FLOWER CLOSURE

Moisture at night could genuinely represent a problem for Namaqualand plants with sensitive pollen. The air regularly becomes saturated at night. Relative humidity increases to 100%, while corresponding temperatures reach fairly low values (figure 7). Under these circumstances, net evaporation and condensation are in equilibrium[20]. Condensation on plants will occur when their surface temperature is below dew point temperature, because cool air has a lower water holding capacity than warm air[20]. Since flower temperatures are noticeably lower than ambient conditions in the morning (figure 5 a,b), it is likely that they reach dew point temperature at night. This is confirmed by observations of dew on the outside petals of flowers every morning during the study period in August.

The environmental conditions outlined above are responsible for the common occurrence of dew in Namaqualand [1,10]. The precipitation of moisture is an essential part of the argument that water poses a risk for plants. The measure of relative humidity alone is not sufficient to explain pollen damage. It merely reflects the amount of water vapour in the air, to which most pollen does not appear to be very sensitive. Anthesis, pollen presentation and germination on the stigma in many species are not adversely affected by high humidity[19] or can be enhanced (e.g. in tropical plants used to humid conditions[11]). Thus, it is the direct contact with moisture, in the form of dew droplets or rain, that plays a crucial role in damaging pollen grains.

Yet, how can water damage pollen? The answer to this lies in the physiology and biochemistry of pollen and in its interaction with the stigma. Pollen grains usually develop in the anthers at high humidity[12]. The development of desiccation tolerance, a common feature in many species [3,12,13,23,24,27], occurs in the last few days before

anthesis[12]. During this time, starch within the pollen grains is degraded and sucrose levels increase dramatically[3,12]. The disaccharide stabilises membranes and prevents damage associated with desiccation in cells[3]. Upon dehiscence of the anther, pollen grains are released into comparatively dry surroundings (lower relative humidity at daytime, figure 7) and start drying out. Pollen of some species is already released in a dehydrated state [30]. Once pollination has been successful, pollen grains are stimulated to germinate on the stigma [13,23,24,30]. This involves a series of not yet fully understood interactions between the stigma and pollen[30]. The stigma is responsible for the highly controlled rehydration of the pollen grains[23,30] which causes complex internal rearrangements in pollen grains [30]. Pollen tubes subsequently grow into the style to fertilise ovules. Contact with water can cause premature and uncontrolled rehydration of pollen grains while still on the donor plant[4,11,12]. This is associated with a loss in pollen viability as the interaction with water can mobilise pollen grains to flow out from the exine cavity or cause other damage [4,30]. These effects are a consequence of contact with moisture and cause a reduction in the number of viable pollen grains in a flower.

The processes through which imbibitional damage occurs at the cellular level, vary[3]. Rapid and uncontrolled water uptake by fairly desiccated pollen grains (e.g. at the end of a day) disrupts phospholipid membranes[3, 12]. The resulting leakage of solutes decreases viability and is especially acute at low temperatures (below 15°C) [3]. This highlights the risk of leaving pollen exposed to water at cool night-time temperatures in Namaqualand. Slow and controlled rehydration, as it occurs on the stigma, does not reduce pollen viability[3,11,23]. Apart from leakage, uncontrolled hydration can lead to damage by organic radicals, as shown *Typha latifolia* pollen[21]. During hydration, free radicals are released from a trapped state[21]. These pose a threat as they destabilise membranes and can cause pollen grains to become non-viable. The risks associated with premature and uncontrolled rehydration of pollen grains are serious and can have a negative effect on the reproductive potential of a plant.

In the Namaqualand flora, petal closure (table 1) seems to be the dominant protective mechanism to keep the centre of flowers dry so that pollen is not exposed to rain or dew. Humidity within flowers of the study plants could not be measured due to the lack of appropriately small humidity probes. The potential for dew formation within the flowers was therefore not determined. An examination of the inside of flowers in the morning, however, revealed that the centres were dry, despite the presence of dew outside.

Evidence that flower closure is an effective protective mechanism against moisture emerges from the pollen experiments conducted. Four species (*C. spongiosum, L. hoorlianus, A. merxmuelleri* and *Cephalophyllum* sp., figure 8) show considerable damage of pollen grains following artificial hydration. In these plants, sensitivity to moisture was suspected as they close their petals (table 1) very tightly at night. This protective mechanism makes sense in 'mesemb' species (members of the Mesembryanthemaceae) due to their floral morphology. When open, their insect-pollinated flowers are roughly saucer-shaped, with petals that are much longer than the stamens[10]. The entire centre is covered in a dense array of exposed stamens, thereby giving them the name of 'stamen carpet flowers' [10]. Copious amounts of dry powdery pollen are present (figure 2), which would be exposed to the environment at night if petals remained in the open position. Dew could gather in flowers and rain would certainly wash away much of the pollen.

The abundance of powdery pollen in the stamen-carpet flowers has been suggested to indicate the importance of wind pollination, in addition to insects. This argument is backed by observations that seed-set was low in plants where pollen had been wetted during watering[10]. The explanation given for low seed-set is that wetted pollen can no longer be transferred in the air and thus pollination events are rare[10]. In view of the results in this study, an alternative interpretation (apart from the absence of insects)

can be proposed for these observations. As pollen of the 'mesemb' species is susceptible to damage by water (figure 8) low-seed set can be attributed directly to pollen damage caused by wetting flower heads. Water may therefore, affect both male fitness (pollen viability) and female fitness (seed-set) in flowers. The latter may simply be a result of reduced male fitness, but may also point to water influencing stigma receptivity. Experiments on *Brassica oleracea* [30] show that water on the stigma just prior or after pollination greatly reduces or prevents successful fertilisation. This is partly attributable to the detrimental effect of water on pollen grains which may not be able to germinate naturally. In addition, however, the pollen-stigma interaction seems to be modified by water which also causes a disorganisation of the stigma surface[30].

The results obtained in the natural field experiment on *C. spongiosum* and *L. hoorlianus* independently confirm the outcome and interpretation of artificial hydration experiments in these species (figure 8). The higher percentage of damaged pollen in the artificial treatment (figure 8) can be attributed to the length of the hydration period. Pollen in the field was exposed to the environment for approximately 12 hours (overnight) but in the artificial treatment pollen remained in water for 36 hours. This is probably too long and pollen damage in artificial hydration is overestimated. As conditions in the natural experiment are more appropriate, these results should be used as a guideline. Pollen damage was still significant (figure 8) and can most probably be linked to contact with water overnight. Other unknown factors cannot be completely eliminated as causes for damage, although results of the controlled artificial water treatment highlight moisture as the responsible factor. Rain or dew thus seem to be the greatest problem at night. A comparison of open and control flowers (figure 8) in field experiments shows that, whatever the limiting factor, pollen in open flowers is more damaged than in closed flowers. So flower closure represents mechanism for protecting pollen from adverse conditions at night.

Pollen damage after exposure to water is also considerable in *A. merxmuelleri* (figure 8). As a member of the Asteraceae, the 'flowers' of this species are, strictly speaking, inflorescences. The composite flower heads encompass an aggregation of small individual florets in the centre (disc-florets) surrounded by ray florets. A few new disc florets open every day to expose anthers and shed pollen. At night they close again. Each floret lasts two or three days (personal observations) and unopened ones remain tightly closed. This strategy, in itself, already seems to offer protection to pollen. Yet, in addition, *A. merxmuelleri* closes its petals (ray florets). Thus, two mechanism seem to keep pollen from contact with moisture. Each acting by itself may not be effective enough, so that *A. merxmuelleri* relies on both. Alternatively, only one may be responsible for protecting pollen. Petal closure could be redundant (as an evolutionary hang-over) or have a function other than protecting pollen. On the other hand, floret closure may not be an efficient way of keeping pollen safe. Field experiments using this species would be interesting to clarify these points.

In contrast with the other species, Hydration had no significant impact on pollen of *O. oppositifolium and O. cylindrica* (figure 8). The floral structure in these species resembles that of *A. merxmuelleri*, except that they roll their petals backwards at night (table 1, figure B appended). The reason for this behaviour is unknown but may be associated with other selective pressures, such as herbivore avoidance.
The results for *O. oppositifolium and O. cylindrica* are interesting in that they contradict the contention that pollen which is not usually exposed to water should be sensitive to hydration[4]. The pollen grains of these asterid species are not regularly exposed to moisture since they are enclosed in florets overnight. At the same time, water does not affect them. Thus, they can either tolerate the effects of water or they rely on a different way of receiving protection. A germination inhibitor, as in *Primula vulgaris*[7], could be present. The abundant pollenkitt (sticky substance that

allows adhesion of pollen to insects) around pollen of *O. oppositifolium and O. cylindrica* may also ward off moisture due to its lipid base [11].
Consequently, enclosure within florets does not represent a pollen protection mechanism in these species and petal closure above the central disc is obsolete.

It is shown that various mechanisms for protecting pollen may exist within the species grouped under Asteraceae. This distinguishes them from the Mesembryanthemaceae which appear more uniform in their the strategy of petal closure. This may be of taxonomic significance and could be linked to floral morphology. Yet, despite different strategies, there is seems to be unity regarding the driving factor for the mechanisms. They are aimed at keeping pollen from coming into contact with moisture. Protection appears to be successful, as control treatments for most species show far lower numbers of damaged pollen than hydration treatments (figure 8). It would be interesting to quantify the viability of control and hydrated pollen by using a reliable method such as the fluochromatic reaction test [27]. The approach followed in this study of equating pollen damage with loss in viability was, on a whole, adequate owing to the severe nature of the damage.

Alternative hypotheses for flower closure

Moisture as the driving force for flower closure is at the centre of investigation of this study. Yet, alternative hypotheses attempting to explain the behaviour exist. Firstly, flowers may protect reproductive parts from the cold by closing. In view of the mild temperatures (figure 7) in Namaqualand [1,2,15] this is unlikely. Secondly, flowers may close in order to provide refugia for pollinators overnight or in adverse weather. This suggestion is prompted by observations that insects are frequently found in closed flowers, which seem to serve as resting and mating sites. A diverse assemblage of insects, including pollinating and predominantly herbivorous groups, is present in the flowers of several species (unpublished data). Unless there is an advantage associated with housing insects overnight, it is difficult to imagine that flower closure would have

evolved in response to insects. One advantage during mass flowering times would exist, if insects covered in pollen emerged from the flowers of a certain species in the morning and moved directly to another flower of the same species. Flower closure could then represent an adaptation to increase reproductive success. The provision of a refugium would be a reward for the pollinator just as pollen or nectar is.

There may be merit in this hypothesis and it is worth exploring further. Yet, it is also possible that insects simply capitalised on the occurrence of flower closure that developed in response to different pressures. In view of the results in this study, it is likely that the risk of exposing pollen grains to moisture provided the evolutionary thrust to develop the response of flower closure under certain conditions.

The evolutionary significance of flower closure

The loss of reproductive potential due to the effects of moisture on pollination success poses a serious constraint for many plants in Namaqualand. Flower closure is one way of overcoming this constraint. This is especially important, as most of the species in the region depend upon annual flowering, seed set and seedling establishment for persistence[1]. Any loss in reproductive output due to unfavourable environmental conditions, which commonly occur during the flowering season, could jeopardise recruitment. Plants can therefore not risk a reduction in their pollination success by exposing their pollen to moisture. Strong selective pressures would be expected to act on plants to maximise reproductive output. These should drive species of very different groups to evolve mechanisms for the protection of pollen. Flower closure seems to be the most wide-spread strategy for pollen protection. It may be a feature that is easy to evolve.

CONCLUSION

The hypothesis stated in the introduction is supported by the findings of this study. Temperature and moisture emerge as the most important environmental influences on flower closure. These two factors play a fundamental role in Namaqualand as they form part of the unusual selective regime characterising this system. Temperature serves as the cue flower opening and closure. The driving force for the behaviour is the damaging effect of rain and dew on pollen viability. In this light, flower closure is a characteristic of evolutionary importance since it reduces reproductive losses associated with flowering during the wet season. The phenomenon of flower closure is, therefore, a consequence of the selective regime in Namaqualand and adds to the list of unusual biological features within the flora that are attributed to this regime. Further exploration of the system is essential for uncovering the evolutionary and ecological forces that determine the fundamental dynamics in this desert region.

ACKNOWLEDGEMENTS

Thank you to Alan Ellis and Richard Cowling for many ideas and discussion. I greatly appreciate their, Jonathan Colville's and Michael Raine's help in the field and Henry Botha's assistence with the equipment.

REFERENCES

1. **Cowling, R.M., Esler, K.J. & Rundel, P.W.** Namaqualand, South Africa - an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology.* 1998. In press.
2. **Cowling, R.M., Esler, K.J., Midgley, G.F. & Honig, M.A.** 1994. Plant functional diversity, species diversity and climate in arid and semi-arid southern Africa. *Journal of Arid Environments* 27: 141-158.
3. **Crowe, J.H., Hoekstra, F.A. & Crowe, L.M.** 1992. Anhydrobiosis. Annual Review of Physiology 54: 579-599.
4. **Dafni, A.** 1996. Autumnal and winter pollination adaptations under Mediterranean conditions. *Bocconea* 5: 171-181.
5. **Dhingra, H.R. & Varghese, T.M.** 1984. Effect of salt stress on viability, germination and endogenous levels of some metabolites and ions in Maize (*Zea mays* L.) pollen. *Annals of Botany* 55: 415-420.
6. **Eisikowitch, D., Gat, Z., Karni, O. Chechik, F. & Raz, D.** 1992. Almond blooming under adverse conditions. A compromise between various forces. Pp 234-240 in: Proceedings of the sixth International Conference on Mediterranean Climate Ecosystems (MEDECOS VI) - Athens.
7. **Eisikowitch, D. & Woodell, S.R.J.** 1975. The effect of water on pollen germination in two species of Primula. *Evolution* 28: 692-694.
8. **Fagerberg, W.R. & Allain, D.** 1991. A quantitative study of tissue dynamics during closure in the traps of Venus flytrap, *Dionaea muscipula. American Journal of Botany* 78: 647-657.
9. **Fleurat-Lessard, P.** 1988. Structural and ultrastructural features of cortical cells in motor organs of sensitive plants. *Biological Review* 63: 1-22.
10. **Hartmann, H.E.K.** 1991. Mesembryanthema. *Contributions from the Bolus Herbarium* 13: 75-157.
11. **Henny, R.J.** 1985. In vivo pollen germination of *Aglaonema* affected by relative humidity. *HortScience* 20: 142-143.

12. **Hoekstra, F.A. & van Roekel, T.** 1988. Desiccation tolerance of *Papaver dubium* L. pollen during its development in the anther. *Plant Physiology* 88: 626-632.

13. **Kearns, C.A & Inouye, D.W.** 1993. Techniques for Pollination Biologists. University Press of Colorado. Colorado.

14. **Knuth, P.** 1905. *Knuth's Handbook of Flower Pollination.* Leipzig.

15. **Milton, S.J., Yeaton, R.I., Dean, W.R.J. & Vlok, J.H.J.** 1997. Succulent Karoo, pp. 131-166. In: Cowling, R.M., Richardson, D.M. & Pierce, S.M. (eds.). *The Vegetation of Southern Africa.* Cambridge University Press. Cambridge.

16. **Noy-Meir, I.** 1973. Desert ecosystems: environment and producers. *Annual Review of Systematics and Ecology* 4:25-51.

17. **Noy-Meir, I.** 1974. Desert ecosystems: Higher trophic levels. *Annual Review of Systematics and Ecology* 5:195-214.

18. **Percival, M.** 1949. Pollen presentation and pollen collection. *New Phytologist* 49: 40-64.

19. **Percival, M.** 1955. The presentation of pollen in certain angiosperms and its collection by *Apis mellifera. New Phytologist* 54: 353-368.

20. **Preston-White, R.A. & Tyson, P.D.** 1988. *The Atmosphere and Weather of Southern Arica.* Oxford University Press. Cape Town.

21. **Priestley, D.A., Werner, B. G., Leopold, C.A. & McBride, M.B.** 1985. Organic free radical levels in seeds and pollen: the effects of hydration and ageing. *Physiologia Plantarum* 64: 88-94.

22. **Rutherford, M.C. & Westfall, R.H.** 1986. Biomes of Southern Africa - an objective categorisation. Memoirs of the Botanical Survey of South Africa 54: 1-98.

23. **Shivanna, K.R. & Johri, B.M.** 1985. The Angiosperm Pollen - Structure and Function. Wiley Eastern. New Dehli.

24. **Shivanna, K.R. & Rangaswamy, N.S.** 1992. *Pollen Biology - A Lab Manual.* Springer Verlag. Berlin.

25. **Stirton, C.H.** 1983. Nocturnal petal movements in the Asteraceae. *Bothalia* 14: 1003-1006.

26. **Struck, M.** 1994. Flowering phenology in the arid winter-rainfall region of Southern Africa. *Bothalia* 24: 77-90.

27. **Stone, J.L., Thomson, J.D. & Dent-Acosta, S.J.** 1995. Assessment of pollen viability in hand-pollination experiments: a review. *American Journal of Botany* 82: 1186-1197.

28. **Van den Honert, R.** 1997. *Intermediate Statistical Methods for Business and Economics.* University of Cape Town Press. Cape Town.

29. **Zar, J.H.** 1996. *Biostatistical Analysis* (3rd. ed.). Prentice Hall International. London.

30. **Zuberi, M.I. and Dickinson, H.G.** 1985. Modification of the pollen-stigma interaction in *Brassica oleracea* by water. *Annals of Botany* 56: 443-452.

31. **Seymour, R.S.** 1997. Plants that can warm themselves. *Scientific American* 277: 90-95.

FIGURES AND TABLES
in the 'Methods' section:

Figure 1. Location map showing Namaqualand (Succulent Karoo Biome) and the approximate position of the study area into which sites 1 - 4 (see figure A, appended) fall. The delimitation of the bioregion comprising coastal strandveld is also displayed. Map redrawn from 1.

Figure 2. *Cephalophyllum spongiosum* flowers early in the morning (1a) with dew droplets visible on the petals of the closed flower and on the vegetative plant parts. In the open state at midday (1b) the flower has a bee-fly sitting amongst the stamens. Note the carpet-flower appearance[10] and the abundance of yellow, powdery pollen (1b).

Table 1. Characteristics of study species belonging to the Asteraceae, Oxalidaceae and Mesembryanthemaceae. Flower size refers to the mean diameter of ten open flowers per species. (Abbreviations: cm - centimetres, m - metres). Note that the flower head of the first two species of the Asteraceae is in cross-section while the side view
is displayed for other species. The illustrations only serve to show the type of petal closure encountered.

In the 'Results' section:

Figure 3. Trends in flower closure (% flowers closed) and ambient temperature (°C) graphed over 4 consecutive days in July 1998. Note that only four out of the 9 species that were monitored are illustrated.

Figure 4. Relationship between flower closure (% flowers closed) of eight individual species and ambient temperature (°C). The non-linear Boltzmann sigmoid equation $Y = Bottom + (Top - Bottom) / (1 + \exp((V50 - X) / slope))$ was used to model data for all species. The regression coefficient r^2 and the temperature threshold at which 50% of

flowers are open ($T_{50\%}$) are given for each plot. Values for the parameters bottom, top and slope are added as an appendix for each species (see table A).

Figure 5. Trends in flower temperature (°C) of *Cephalophyllum spongiosum* and *Othonna cylindrica* and in ambient temperature (°C) over two days (5-6/8) in August 1998 (a, b). Corresponding patterns of flower closure are illustrated beneath (c, d).

Figure 6. Relation between mean flower temperatures (°C) in *Othonna cylindrica* (n=30) and *Cephalophyllum spongiosum* (n=32) and ambient temperatures (°C) as described by the Stefan Boltzmann sigmoid equation. Parameters of the regression are shown (r^2, $T_{50\%}$) with additional statistics appended (Table A). Note that results of the Spearman Rank Order test for correlations (r_s and p) are also displayed on the plots.
Data stems from 16 occasions on two days (day 2 and 3) in August 1998. Each point refers to one ambient temperature measurement and the mean of temperature measurements of n flowers. (Abbreviations: r_s - Spearman rank coefficient of correlation, r2 - coefficient of determination of the non-linear curve, T50% - temperature where 50% of flowers are open).

Figure 7. Trend in relative humidity and ambient temperature over three days and nights (4-6/8/98). Note the inverse relationship of the two variables. Relative humidity reaches the 100% level for several hours overnight and the minimum temperature of 6°C. The graph is based on continuous readings taken by an Amtec thermohydrograph, situated at site 4 (figure A).

Figure 8. Intact and damaged pollen grains (% of total counted per treatment in each species) for control and hydrated pollen in six species. Chi-squared values and associated probabilities are displayed on each graph. A significant p-value indicates that the frequency of damaged hydrated pollen is significantly higher than that of damaged control pollen. Frequency values corresponding to percentages of pollen grains are appended (Table B).

Figure 1

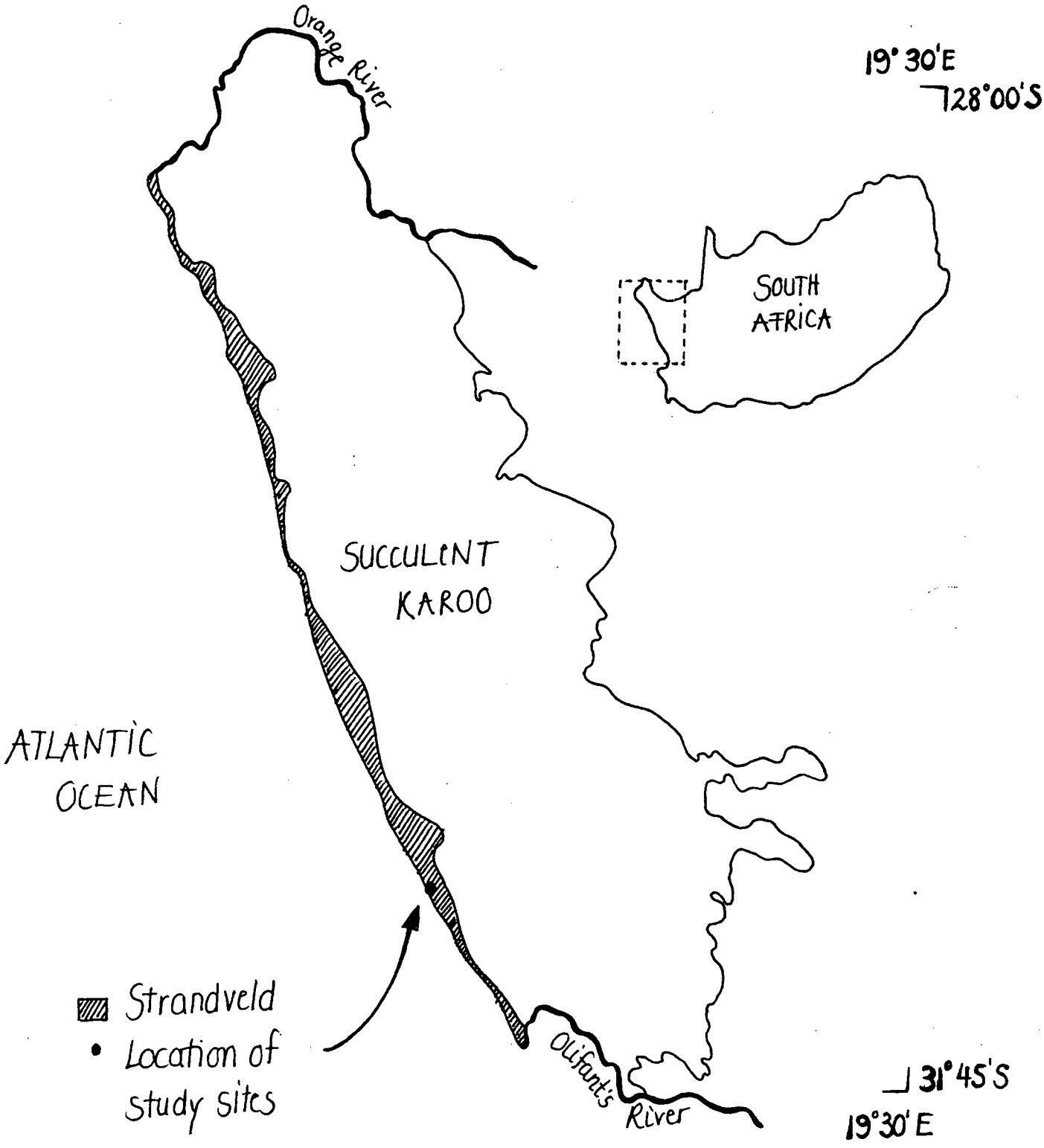

Orange River
19° 30'E
28°00'S
SOUTH AFRICA
SUCCULENT KAROO
ATLANTIC OCEAN
Strandveld
Location of study sites
Olifant's River
31°45'S
19°30'E

Figure 2

2b)

2a)

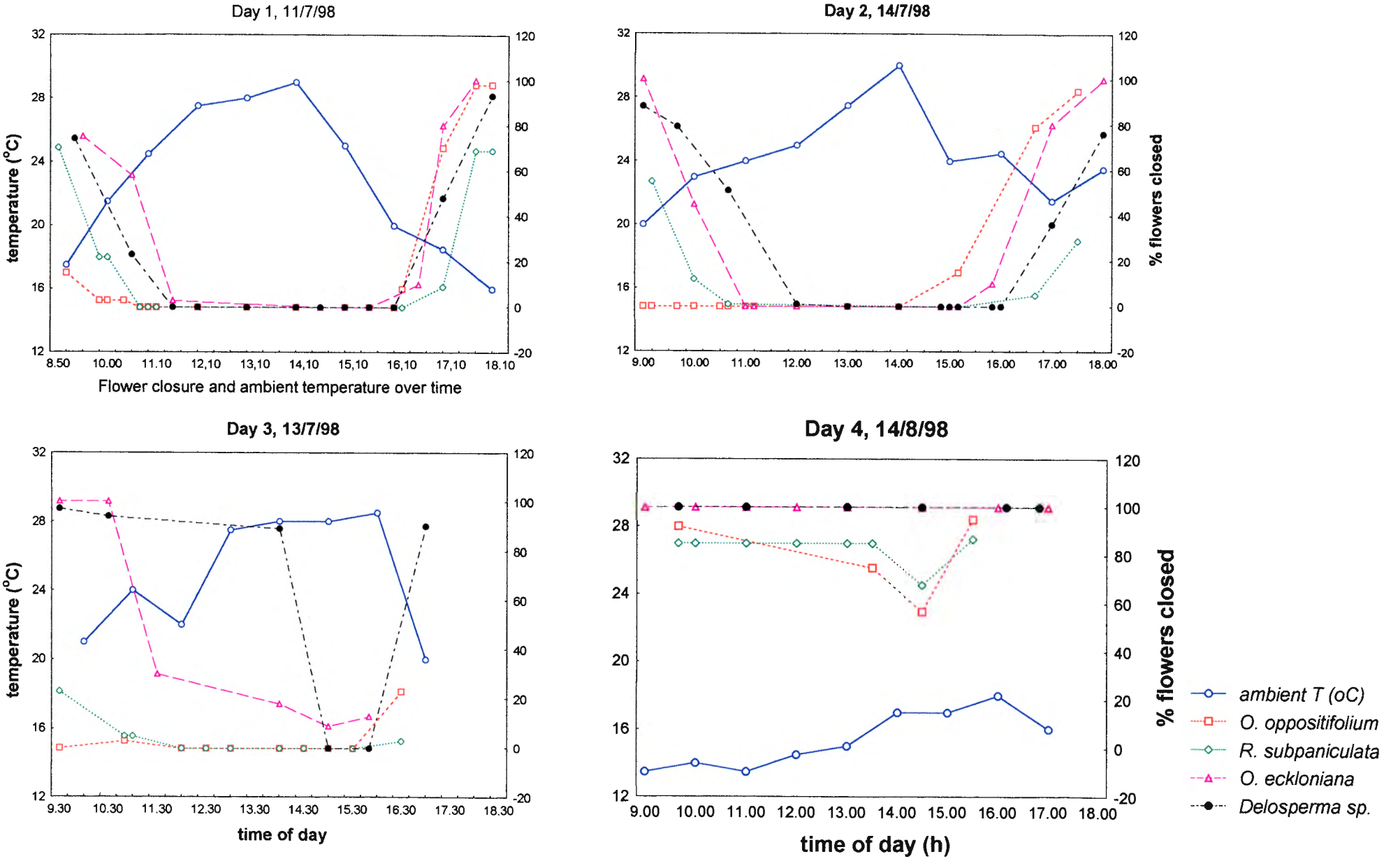
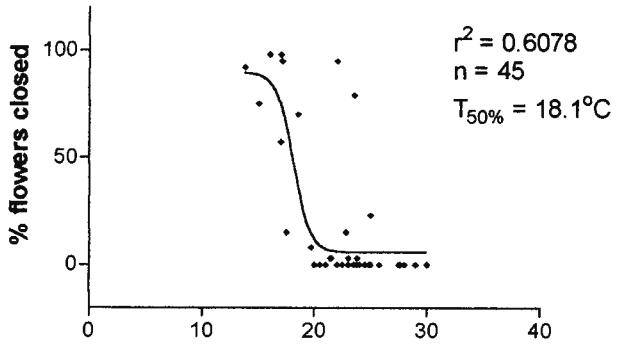

Day 1, 11/7/98
temperature (°C)
Flower closure and ambient temperature over time
Day 2, 14/7/98
% flowers closed
Day 3, 13/7/98
time of day
Day 4, 14/8/98
time of day (h)
% flowers closed
ambient T (oC)
O. oppositifolium
R. subpaniculata
O. eckloniana
Delosperma sp.

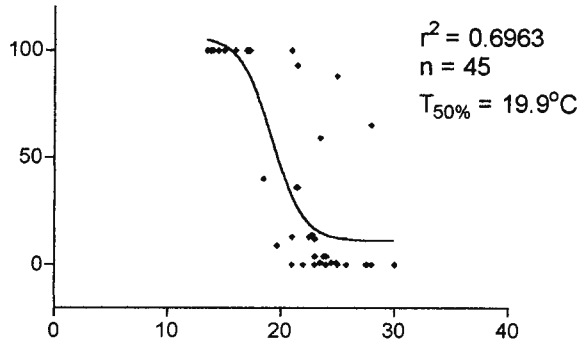
Figure 3

Figure 4

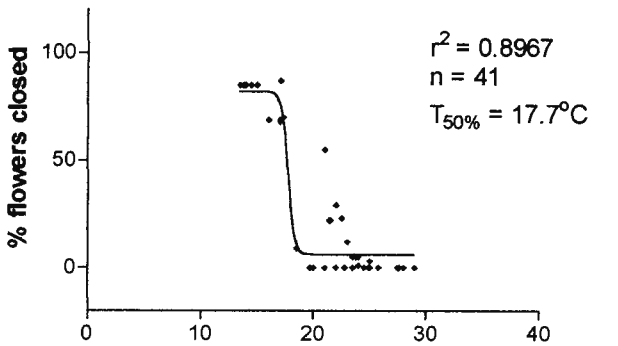
Osteospermum oppositifolium



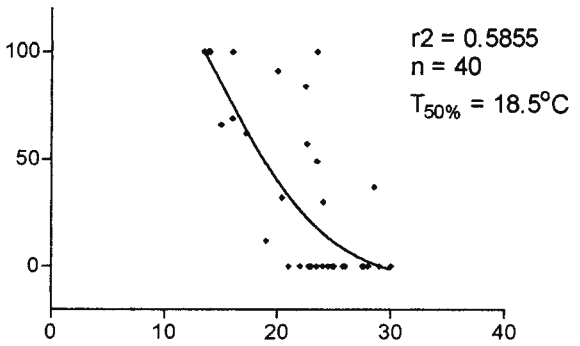
Othonna cylindrica



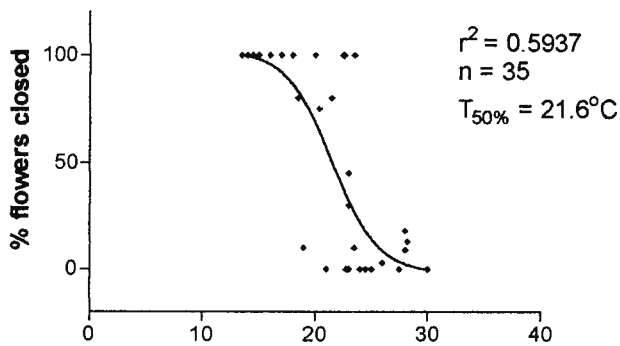
Ruschia subpaniculata



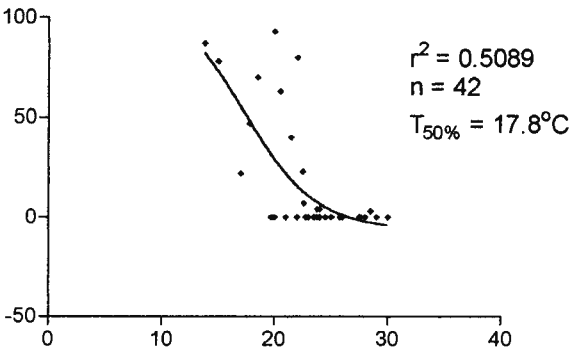
Lampranthus hoorlianus



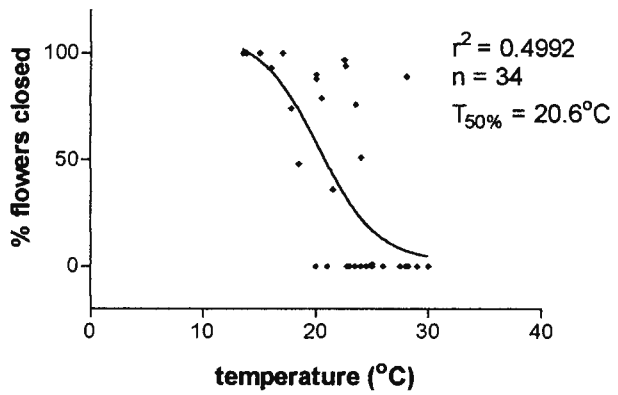
Oxalis eckloniana



Didelta carnosa* var. *tomentosa



***Delosperma* sp.**



Cephalophyllum spongiosum

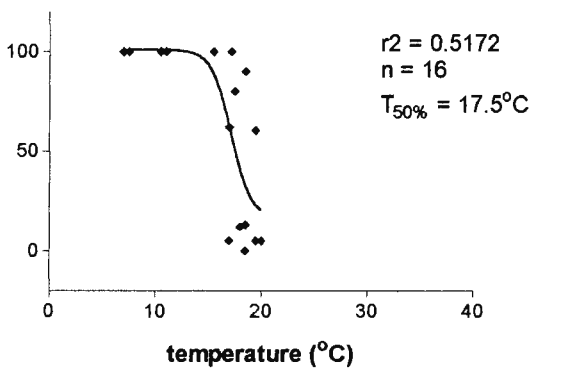


Figure 5

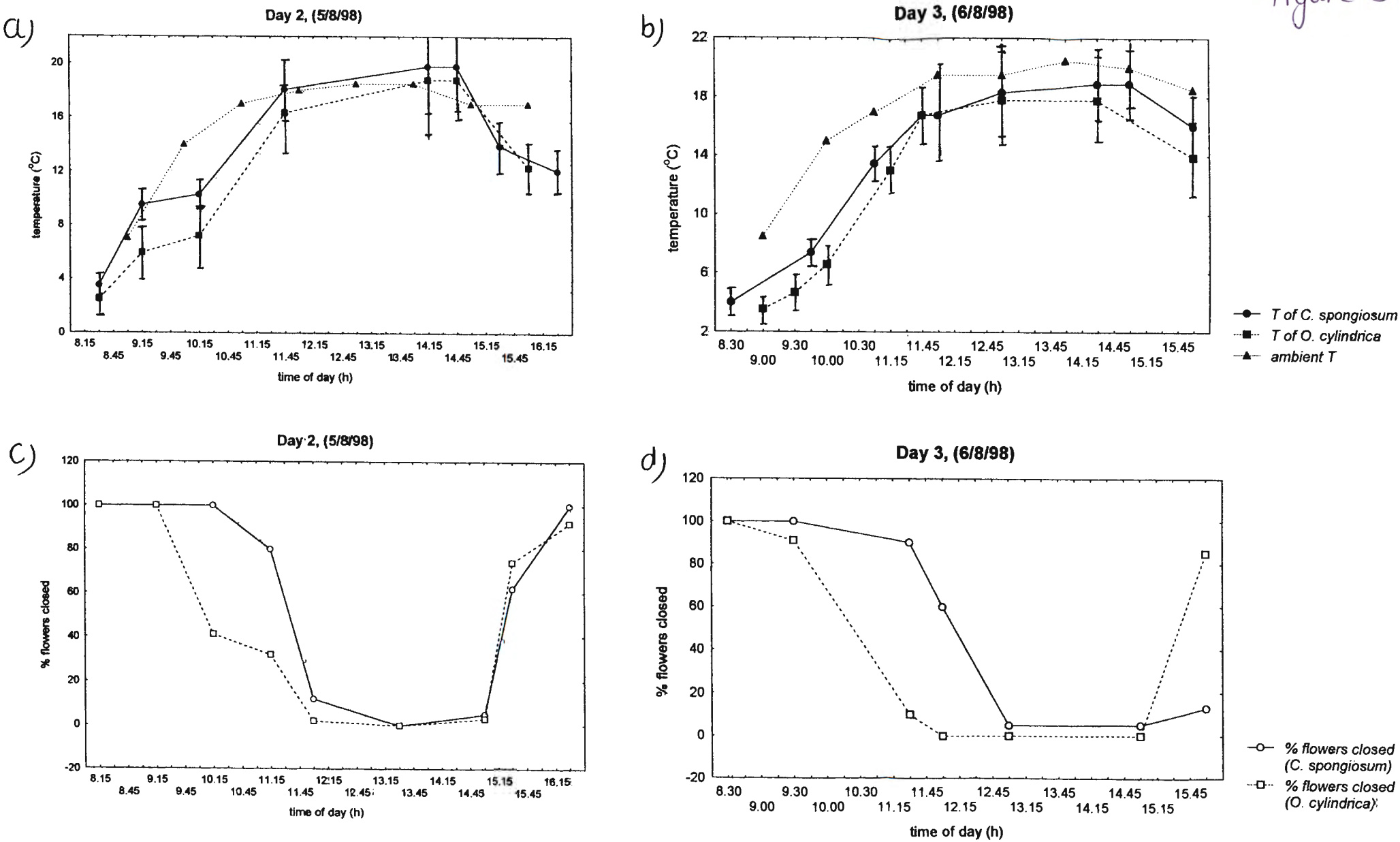
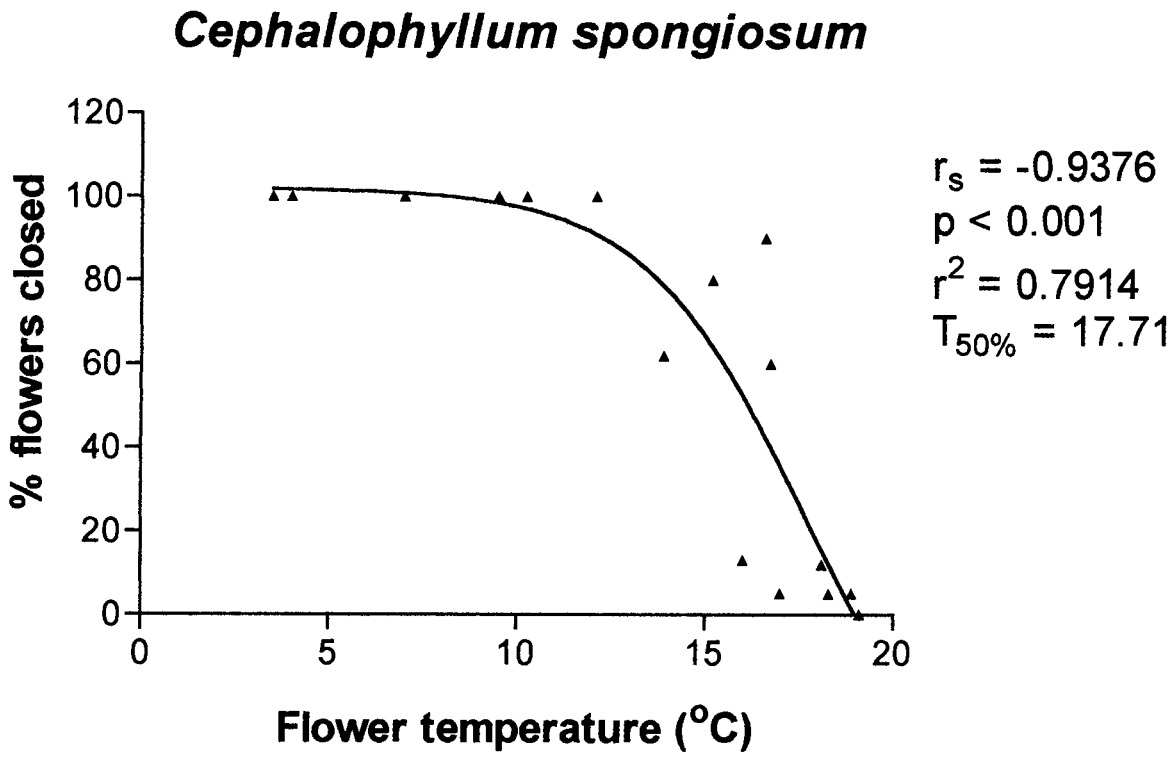

a)
Day 2, (5/8/98)
temperature (°C)
time of day (h)
b)
Day 3, (6/8/98)
temperature (°C)
time of day (h)
T of C. spongiosum
T of O. cylindrica
ambient T
c)
Day 2, (5/8/98)
% flowers closed
time of day (h)
d)
Day 3, (6/8/98)
% flowers closed
time of day (h)
% flowers closed (C. spongiosum)
% flowers closed (O. cylindrica)

Figure 6


Cephalophyllum spongiosum
% flowers closed
120
100
80
60
40
20
0
0
5
10
15
20
Flower temperature (°C)
r_s = -0.9376
p < 0.001
r^2 = 0.7914
$T_{50\%}$ = 17.71

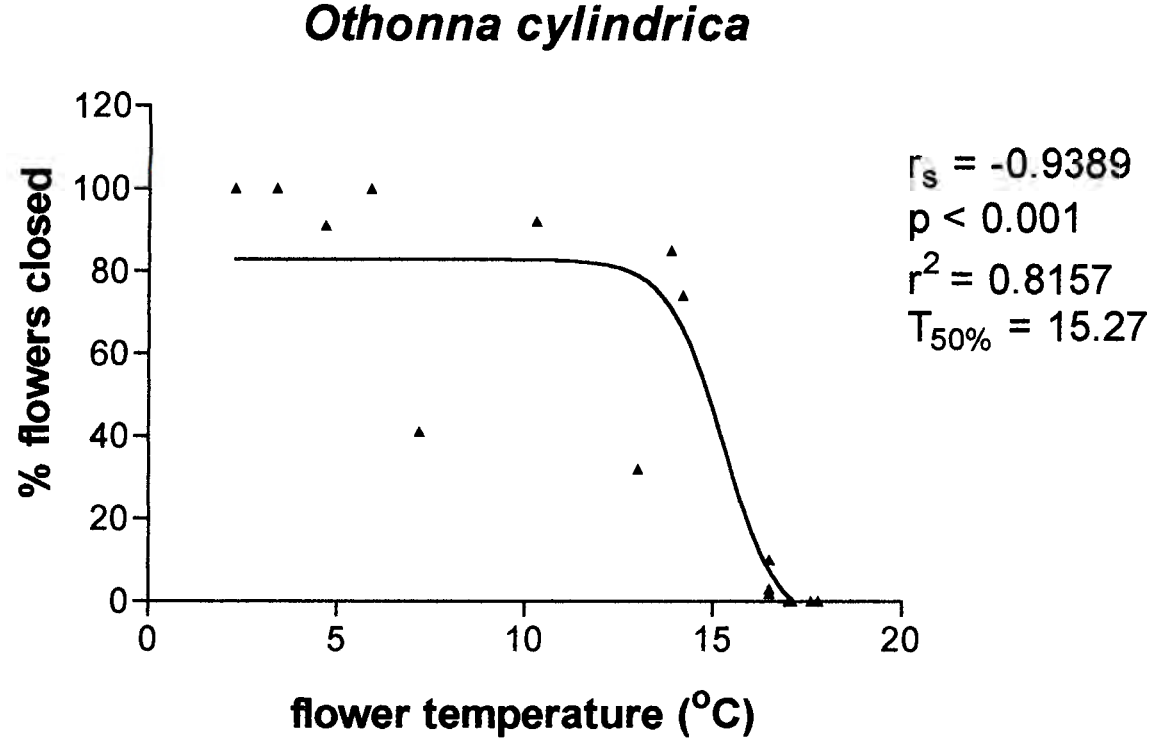

Othonna cylindrica
% flowers closed
120
100
80
60
40
20
0
0
5
10
15
20
flower temperature (°C)
r_s = -0.9389
p < 0.001
r^2 = 0.8157
$T_{50\%}$ = 15.27

Figure 7

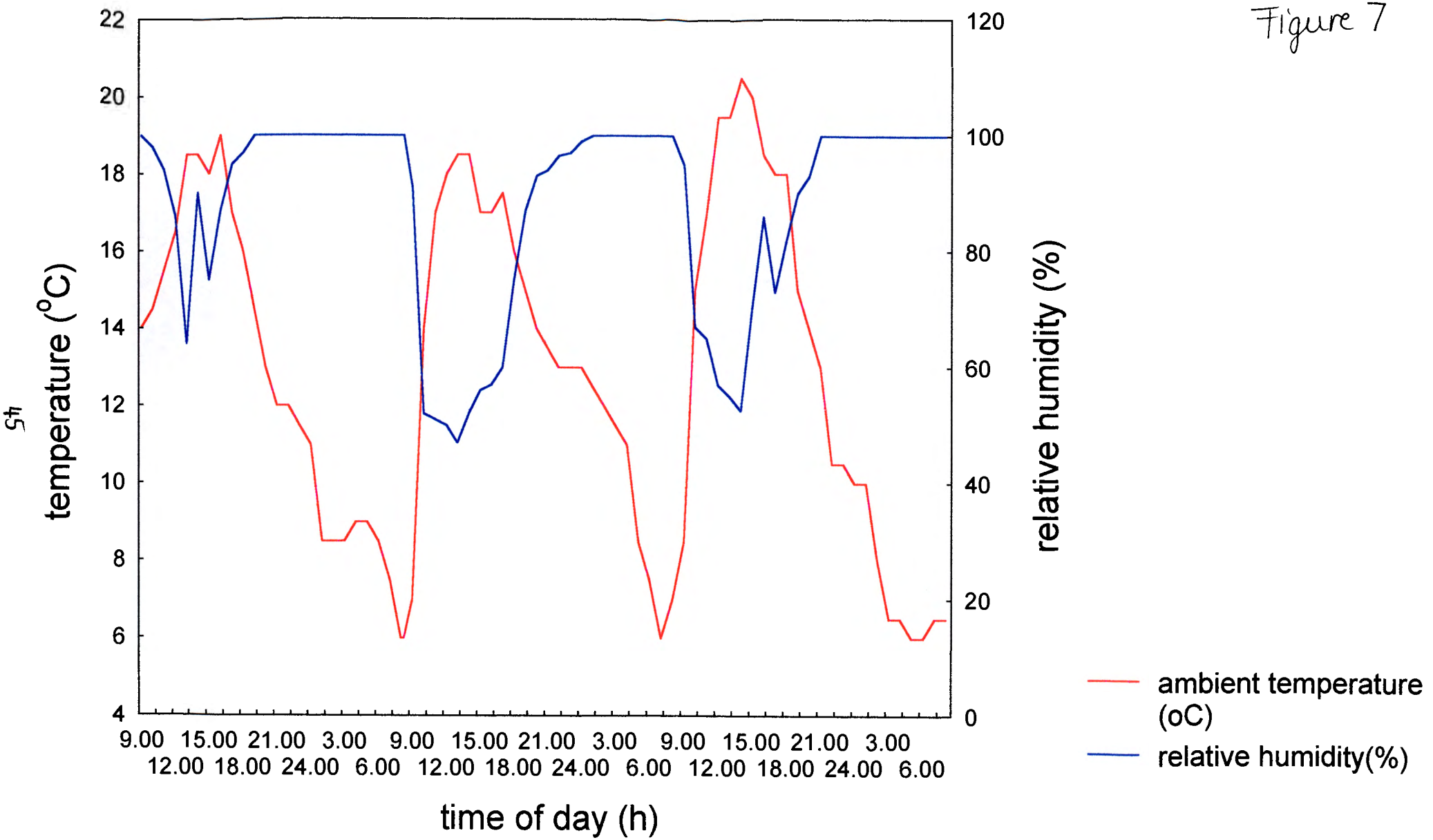
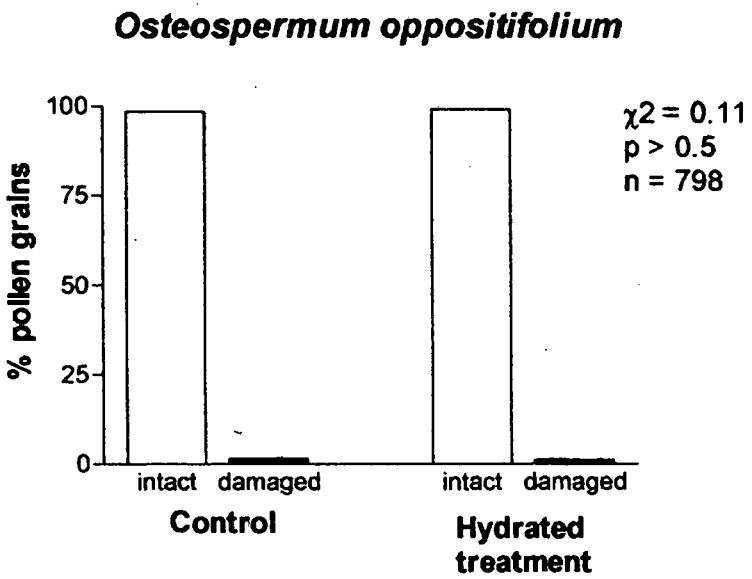
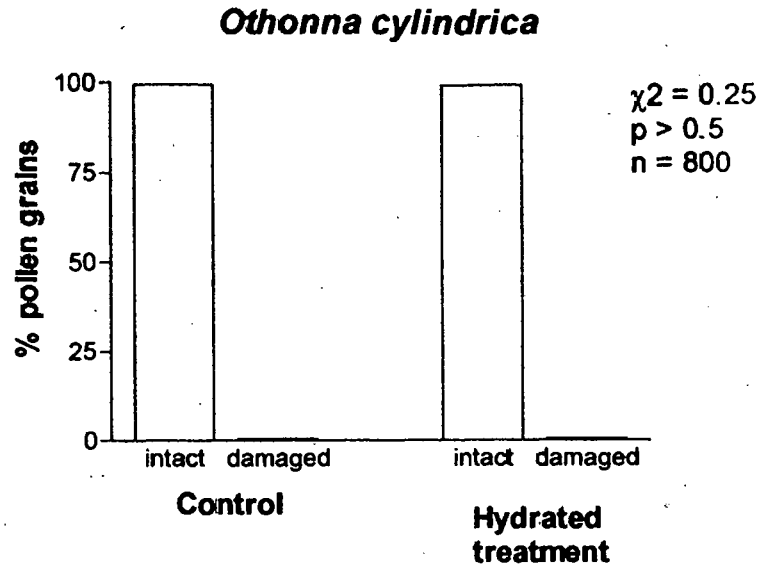
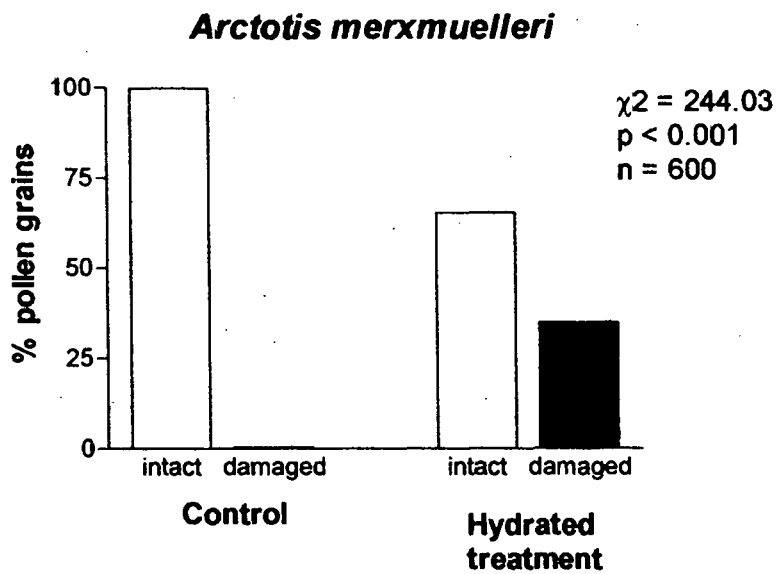

temperature (°C)
relative humidity (%)
time of day (h)
ambient temperature (oC)
relative humidity(%)
22
20
18
16
14
12
10
8
6
4
120
100
80
60
40
20
0
9.00 15.00 21.00 3.00 9.00 15.00 21.00 3.00 9.00 15.00 21.00 3.00
12.00 18.00 24.00 6.00 12.00 18.00 24.00 6.00 12.00 18.00 24.00 6.00

Figure 8


Osteospermum oppositifolium
% pollen grains
100
75
50
25
0
intact
damaged
intact
damaged
Control
Hydrated treatment
χ2 = 0.11
p > 0.5
n = 798


Othonna cylindrica
% pollen grains
100
75
50
25
0
intact
damaged
intact
damaged
Control
Hydrated treatment
χ2 = 0.25
p > 0.5
n = 800


Arctotis merxmuelleri
% pollen grains
100
75
50
25
0
intact
damaged
intact
damaged
Control
Hydrated treatment
χ2 = 244.03
p < 0.001
n = 600

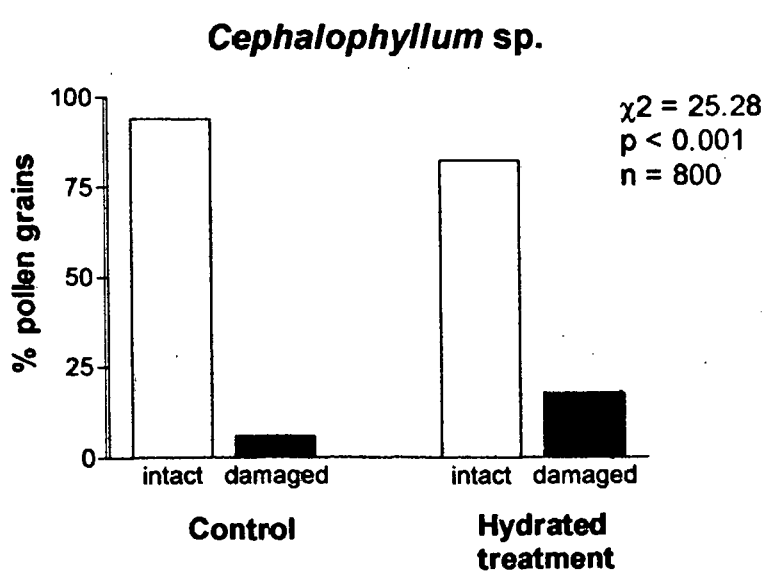
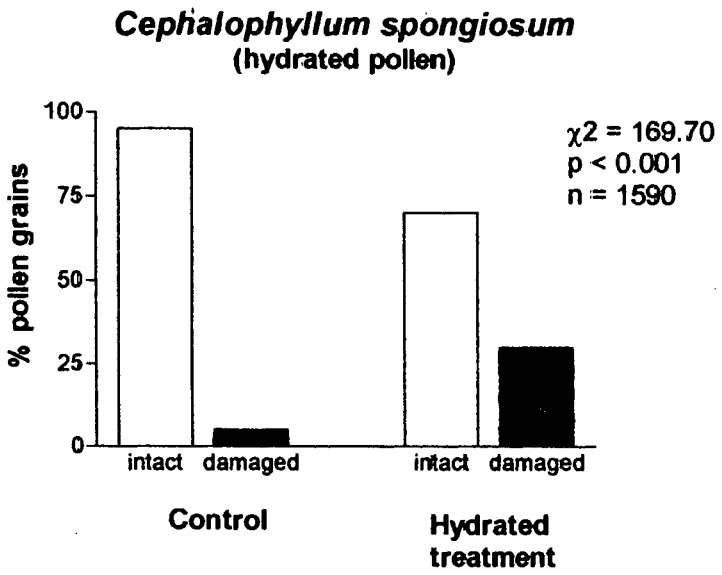
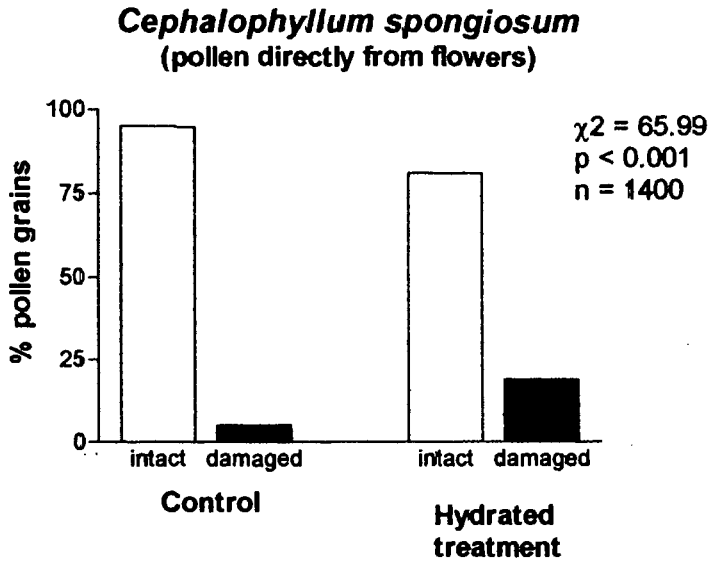
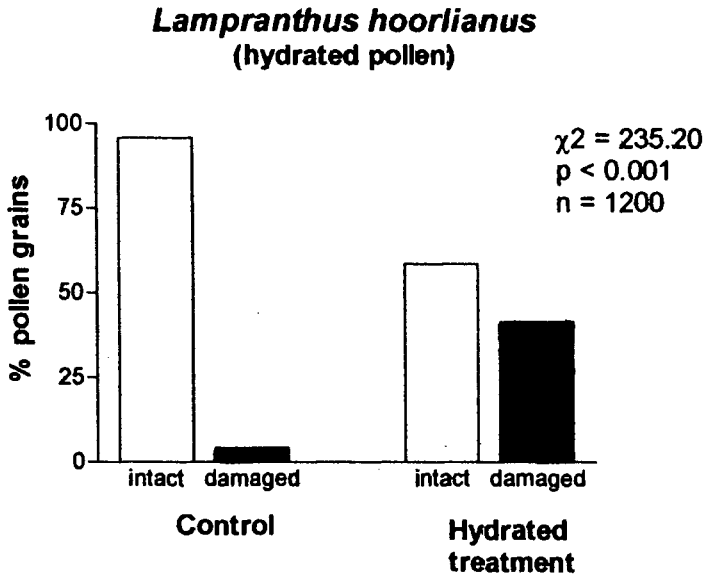

Cephalophyllum sp.
% pollen grains
100
75
50
25
0
intact
damaged
intact
damaged
Control
Hydrated treatment
χ2 = 25.28
p < 0.001
n = 800

Figure 8


Cephalophyllum spongiosum
(hydrated pollen)
% pollen grains
100
75
50
25
0
χ2 = 169.70
p < 0.001
n = 1590
intact
damaged
intact
damaged
Control
Hydrated treatment


Cephalophyllum spongiosum
(pollen directly from flowers)
% pollen grains
100
75
50
25
0
χ2 = 65.99
p < 0.001
n = 1400
intact
damaged
intact
damaged
Control
Hydrated treatment


Lampranthus hoorlianus
(hydrated pollen)
% pollen grains
100
75
50
25
0
χ2 = 235.20
p < 0.001
n = 1200
intact
damaged
intact
damaged
Control
Hydrated treatment

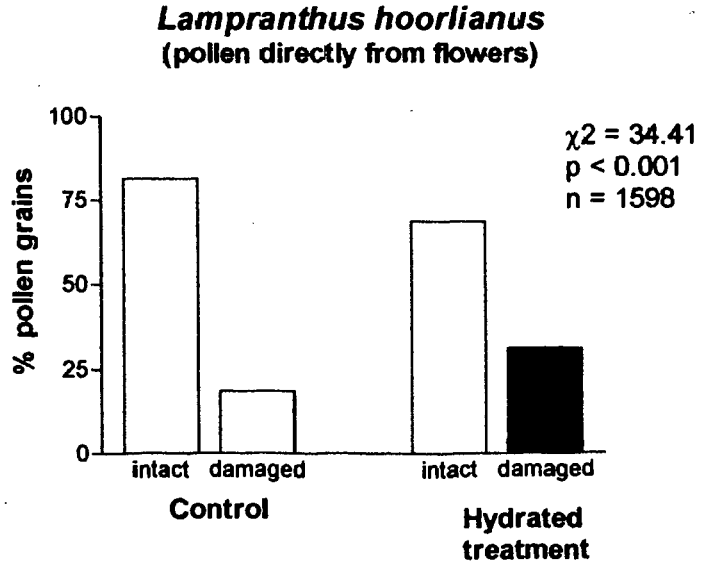

Lampranthus hoorlianus
(pollen directly from flowers)
% pollen grains
100
75
50
25
0
χ2 = 34.41
p < 0.001
n = 1598
intact
damaged
intact
damaged
Control
Hydrated treatment

Table 1.

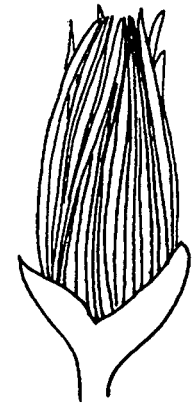
Study species of three different families	Growth form (height in metres)	Leaf succu-lence	Flower size (in cm)	Position of petals in closed flower
Asteraceae:				
Osteospermum oppositifolium	shrub (0.8 - 1.2 m)	yes	4.5	
Othonna cylindrica	shrub (0.3 - 1.0 m)	yes	2.2	
Arctotis merxmuelleri	herbaceous (0.1 - 0.2 m)	no	5.3	
Didelta carnosa var. *tomentosa*	herbaceous (0.1 - 0.2 m)	no	5.4	
Mesemryanthemaceae:				
Cephalophyllum spongiosum	low creeper (0.15 - 0.3 m)	yes	10.0	
Cephalophyllum sp.	low creeper (0.15 - 0.25 m)	yes	4.6	
Lampranthus hoorlianus	shrub (0.5 - 1.0 m)	yes	5.1	
Ruschia subpanuculata	dwarf shrub (0.15 - 0.25 m)	yes	2.0	
Delosperma sp.	dwarf shrub (0.2 - 0.3 m)	yes	1.7	
Oxalidaceae:				
Oxalis eckloniana	herbaceous annual (0.02 m)	no	2.1	

Table 2

Dependent variable	Independent variables	Multiple r2	Adjusted r2	F ratio	p-level (alpha=0.05)
Cephalophyllum spongiosum					
1. Flower T	ambient T	0.9051	0.9120	114.4358	< 0.001
	wind	0.9256		3.0229	< 0.5
2. Flower T	ambient T	0.9051	0.8972	114.4358	< 0.001
Othonna cylindrica					
Flower T	ambient T	0.8734	0.8660	82.8054	< 0.001
	radiation	0.8866		1.2813	< 0.5

* **Independent variable list:**
Ambient temperature (°C)
Wind (m/s)
Radiation (Q. Ir. *10 μ mol/m^2/s)
Relative humidity (% data, arcsin transformed)

APPENDIX

Headings For Figures And Tables:

Figure A. Sites 1 to 4 where the study was conducted. In July 1998 (11 - 14) flowers of different species were monitored at site 1 (Aa), located approximately 200 metres from the sea, at site 2 (Ab), 900 metres from the sea and with low strandveld vegetation and at site 3 (Ac), furthest inland and characterised by medium strandveld vegetation. In August 1998 (5 - 6) *Cephalophyllum spongiosum* and *Othonna cylimdrica* were studied at site 4 (Ad), located 400 metres from the sea.

Figure B. *Othonna cylindrica* shrub with flowers in a closed state (Ba) with the petals rolled backwards exposing the disc-florets and in an open state (Bb) where the petals are fully extended.

Figure C. The course of wind strength (m/s) on two days (5-6/8) in August 1998. Note that wind strength increases notably during the day.

Table A. Additional parameters delimiting the non-linear Boltzmann sigmoid regression curves (figure 4) modelling flower closure (%) versus 1. ambient temperature and 2. flower temperature (°C). Top and bottom refer to the upper and lower limits of each curve where it levels out into a plateau. Slope defines the slope of the entire regression line in each case.

Table B. Frequencies of intact and damaged pollen in control and hydration treatments for six species. Note that two experiments were conducted in *C. spongiosum* and *L. hoorlianus*: 1. Pollen was placed in water (artificial hydration) and 2. Flowers were prevented from closing overnight and 'hydrated' pollen was obtained from these the next morning. Means and variances of counts on separate flowers are given below the table for *C. spongiosum* and *L. hoorlianus.*

Figure A

Aa)

Ab)

Figure A

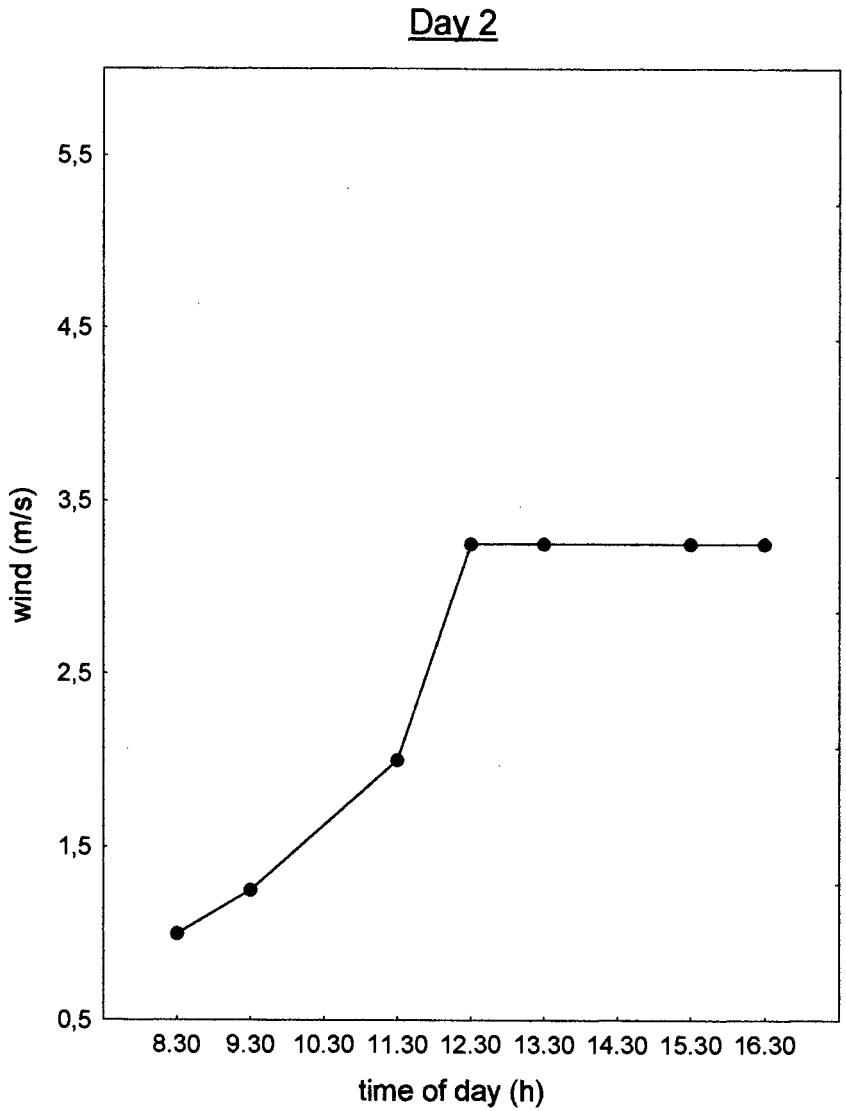
Ac)

Ad)

Figure B

Ba)

Bb)


Day 2
wind (m/s)
5,5
4,5
3,5
2,5
1,5
0,5
8.30 9.30 10.30 11.30 12.30 13.30 14.30 15.30 16.30
time of day (h)

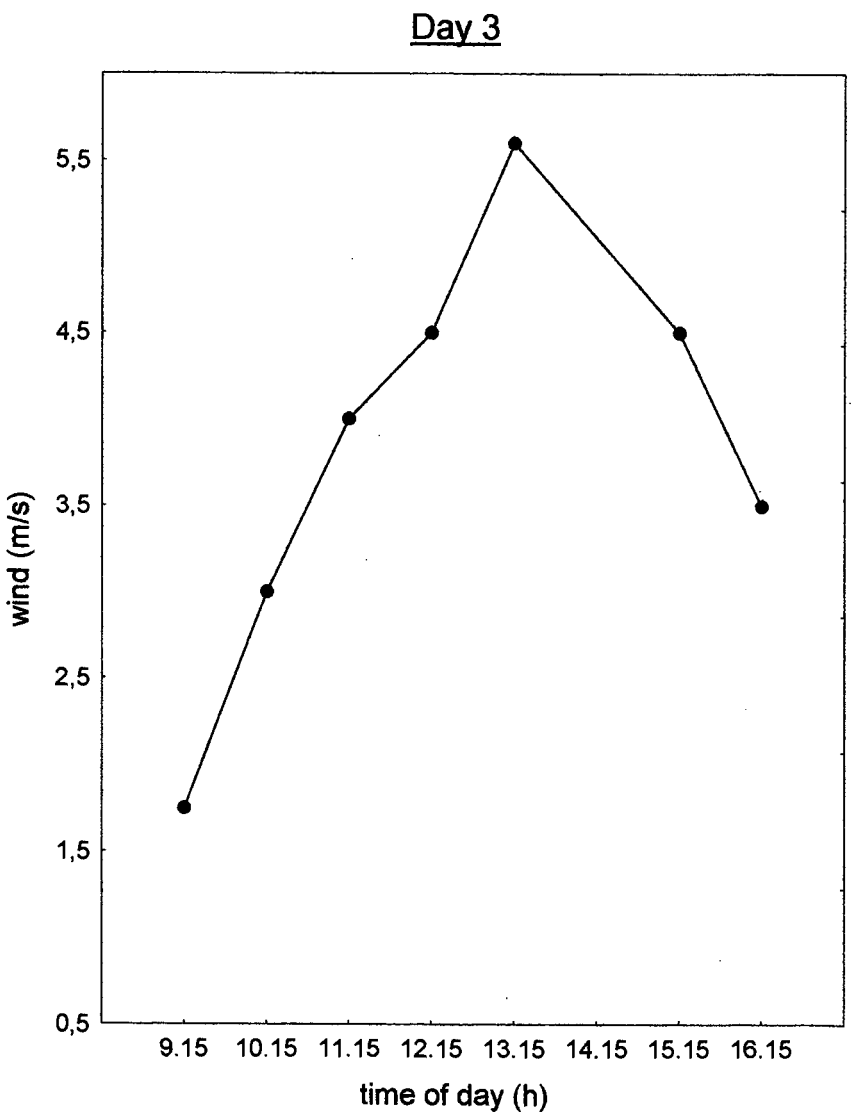

Day 3
wind (m/s)
5,5
4,5
3,5
2,5
1,5
0,5
9.15 10.15 11.15 12.15 13.15 14.15 15.15 16.15
time of day (h)

Figure C

Table A.

Species	Top	Bottom	Slope
1. *Osteospermum oppositifolium*	89.69	5.845	-.07374
Othonna cylindrica	106.6	11.17	-1.386
Ruschia subpaniculata	89.19	6.216	-0.2825
Lampranthus hoorlianus	180.5	-9.432	-4.798
Oxalis eckloniana	102.4	-1.757	-2.040
Didelta carnosa var. *tormentosa*	111.7	-6.153	-3.205
Delosperma sp.	109.3	2.247	-2.616
Cephalophyllum spongiosum	101.0	16.48	-0.9365
2. *Othonna cylindrica*	82.84	-6.544	-0.7363
Cephalophyllum spongiosum	102.0	-56.40	-2.157

Table B.

Species	Control pollen		Hydrated pollen	
	intact	damaged	intact	damaged
O. oppositifolium	392	6	396	4
O. cylindrica	398	2	398	2
1. *C. spongiosum*, hydrated pollen	760	40	554	236
2. *C. spongiosum*, pollen from 3 flowers	760*	40*	487*	113*
1. *L. hoorlianus* hydrated pollen	575	25	351	249
2. *L. hoorlianus* pollen from 4 flowers	650*	148*	549*	251*
Cephalophyllum sp.	376	24	329	71
Arctotis merxmuelleri	598	2	391	209

* Means and (variance) were calculated:

		C. spongiosum (n=3)	*L. hoorlianus* (n=4)
Control -	intact	92.89 (4.39)	81.25 (8.80)
	damaged	7.13 (4.30)	18.75 (8.80)
Hydrated -	intact	68.63 (9.44)	31.13 (9.33)
	damaged	81.25 (7.33)	18.58 (7.44)