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Reproductive biology of *Stomatium bolusiae* (Aizoaceae: Ruschioideae)

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ABSTRACT

Flowers of *Stomatium bolusiae* are self-incompatible. The species exhibits crepuscular and nocturnal anthesis, exploiting two different pollination mechanisms. The structure of the hermaphroditic flower appears not to favour cross-pollination. The stigmata are never exposed to pollinating agents, which gain access to the floral rewards by forcing their way between the anthers. Clogging of the stigmatic surfaces by self-pollen is common. Nocturnal anthesis, concomitant with the nocturnal release of attractants and the offering of rewards, indicates that this species is primarily phalaenophilous and secondarily melittophilous, exhibiting a bimodal pollination system.

INTRODUCTION

Few data exist on the pollination biology or related aspects of Aizoaceae subfamilies Mesembryanthemoideae and Ruschoideae, commonly referred to as mesembs (Vogel 1954; Liede & Hammer 1990; Liede *et al.* 1991; Struck 1995; Hammer 2002; Hartmann 1991, 2002a, 2002b; Juergens 2004; Peter *et al.* 2004; Thiede *et al.* 2011). The only studies in South Africa on visits and possible pollination of mesemb flowers by masarid wasps was conducted by Gess & Gess (1989) and Struck (1994). In diurnal flowering species, the prominence of the bright shiny petals and the open pollen presentation suggest that insects pollinate these flowers (Hartmann 1991).

Available information on anthesis in mesembs is based on studies by Hartmann (1978, 1991) and Hartmann & Dehn (1987), who observed that flowers opening during the day are protandrous, with a very distinctive early male phase, followed by a later female phase. The flowers open repeatedly by basal growth of the androecial elements (Hartmann 1978, 1991). In melittophilous flowers the stigmas are at first shorter than the stamens. During the female phase, the stamens wither and collapse and the elongated stigmas are prominently displayed in the middle of the flower. At this stage the stigmas spread and start to produce a copiously papillate surface, which is more intensively coloured than in the unripe stage. The genus *Stomatium* is regarded as having melittophilous flowers of the recess type (Hartmann 1978, 1991).

Vogel (1954) suggested that visiting insects crawl into this hidden cavity, the depth and width of which may vary between species, in order to reach nectar or pollen. During this activity, pollen is deposited all over the body of the insect.

Stomatium Schwantes is one of numerous genera of Aizoaceae subfam. Ruschioideae (Bittrich & Hartmann 1988; Hartmann 1991). *Stomatium* species are widely distributed through the drier parts of the Western, Northern and Eastern Cape and the central Free State (Smith *et al*. 1998). The genus *Stomatium* comprises 39 species (Smith *et al*. 1998) of which only *S. bolusiae* occurs in the Free State (Chesselet *et al*. 2003). The plants are generally found on rocky outcrops in very shallow soil. The leaves are olive green to drab-grey during favourable periods but take on a reddish or purple colour when stressed, thus blending with the surrounding environment (Smith *et al*. 1998).

The aim of this study was to describe the pollination biology of *S. bolusiae* under natural conditions.

MATERIALS AND METHODS

Study area

The research was conducted in natural veld in an undisturbed part of the Free State National Botanical Garden (2926AA). *S. bolusiae* occurs abundantly on the dolerite outcrops around Bloemfontein. It grows in shallow, gravel-filled depressions and cracks on these outcrops and, during the summer, is exposed to prolonged wet periods after good rains and to drought conditions with extremely high temperatures in between. The vegetation of this area forms part of the Bloemfontein Karroid Shrubland (Gh 8) (Mucina & Rutherford 2006).

Pollen-ovule ratio and pollen viability

Pollen grains were collected from 10 anthers from each of five different flowers representing different ages during the life span of the flowers. These were then separately suspended in 10 ml of a 0.1% (m/v) aniline blue staining solution. After the solutions were thoroughly shaken, pollen grains from four samples of each solution were counted using a haemocytometer. The mean of these four replicates was taken to represent the number of pollen grains per anther. This was then used to determine the standing crop of pollen grains per flower.

Since the ovules are very soft and extremely difficult to dissect from the ovary, seeds of mature fruits were counted and the highest observed value from the ten different mature cross-pollinated capsules, previously unexposed to rain, and excluding the possibility of seed

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loss, was used. This value, together with the average number of pollen grains, was used to obtain an estimate of the pollen/ovule (P/O) ratio. These calculations are based on flowers collected during the 1998/1999 season.

Pollen viability was determined by examining a mixed pollen sample, consisting of pollen from 10 different flowers, using Alexander's viability stain (Alexander 1969) and by means of the fluorochromatic (FCR) test procedure (Heslop-Harrison *et al.* 1984).

Floral behaviour

Ten different individual flowers were monitored for eight days between 16:30 in the afternoon until 04:00 the following morning: (a) to determine the reproductive viable period of flowers from the onset of anthesis and (b) to describe floral behaviour. Observations were recorded at 30-minute intervals. Movement of calyx lobes, petals and anthers were noted. Changes in secretion of nectar glands were also noted.

To test for floral fragrance, different flowers representing the seven days during the life span of the flowers were collected shortly after opening. For each of these days, five flowers were kept in closed vials for a period of three hours. On opening the vials, the odour was evaluated on a subjective scale of 0 (odour absent) to 3 (strong odour) by three different persons. Thereafter, flowers were dissected and the same experiment was conducted on the calyx lobes, pistils, petals and stamen. To localise osmophores, the floral parts were stained with a 0.1% (m/v) neutral red solution for ± five hours (Van Wyk & Lowrey 1988). The presence and availability of nectar and pollen were noted during anthesis.

Pollination experiments and seed set

Experiments were conducted on a single intact flower per plant. A total of 50 flowers were used for each experiment. For microscopic work these flowers were collected 24 hours after having been artificially pollinated and fixed in Carnoy's solution (Samaha *et al*. 1989). Experiments were conducted as described by Bawa (1974), Gunatilleke & Gunatilleke (1984) and Harvey & Braggins (1985). Pollen tube growth and germination were determined in vivo by a technique based upon the fluorescence of callose (Martin 1959; Jefferies & Brain 1984). Pistils were destained with Gillett's Javel commercial bleach containing 3.5% (m/v) sodium hypochlorite, after which the material was thoroughly rinsed in distilled water. After staining with decolourised aniline blue, pollen germination and pollen tube growth were observed using a Zeiss Axioskop equipped with a 100 W high pressure Hg lamp and filter set for UV light excitation. Apomixis was not considered during these experiments as the required emasculation would have damaged the flowers. No flowers were therefore emasculated.

Pollination treatments

Open pollination: flowers were marked and left to test for fruit and seed set.

Autogamy: plants were bagged and left without any manipulation to test for selfing. Due to the leaves covering the fairly short flower stalk and the possibility of damaging the flowers and influencing the experiment, individual flowers were not bagged. Chiffon (a lightweight, balanced, plain-woven sheer fabric) with a mesh size of 250µm × 250µm was loosely stretched over the plant, allowing enough room for floral movement but excluding possible pollinators. It was anchored to the soil with pins. Care was taken to avoid any damage to the plant. Bagging was done in the same way throughout the experiment.

Self-compatibility: flowers were self-pollinated by hand.

Geitonogamy: pollen from one flower was used to pollinate the stigma from another flower on the same plant.

Xenogamy: flowers were crossed with pollen collected from plants 10 m or more from the ones used for the experiment (Waser & Price 1983).

Evaluation of fruit and seed set is based on counts made one month after marking the individual flowers. As it was not clear how many ovules normally abort in the ovaries, an *index of seed set* was used. This was calculated by dividing the actual number of seeds in any capsule by the maximum number of seeds that could be expected to develop under ideal conditions. The latter value represented the highest number of seeds counted in cross-pollinated flowers. Since seed dispersal in *Stomatium* species is initiated by the opening of dry capsules during rain (ombrohydrochory), the risk of losing seeds had to be minimised. Dry capsules, which had not been exposed to rain before, were therefore collected and their seeds counted.

Pollinator behaviour, attractants and rewards

The behavioural pattern of insect visitors to the flowers was observed over a period of five days and nights. Thereafter visitors were collected, killed in 100% ethyl acetate, identified and examined for the presence of *Stomatium* pollen. The placement of pollen on their bodies was noted. Visiting pollinators were identified, counted and visitation rates expressed as number of insects per plant per hour.

Fruit and seed set

To determine the success of seed set throughout the year, 50 mature flowers were marked with threads of cotton every month between September 2001 and August 2002. Ripe capsules were collected ± three to four weeks after being tagged. In total 600 mature capsules were marked and collected during this period. The number of seeds per capsule was determined for each month and compared with the rainfall figures for the same period.

RESULTS

Floral behaviour

The flowers have short stalks and numerous, bright yellow petals. Flowering starts in early spring during August and continues until early winter at the end of May. Plants very seldom flower in mid-winter. During early spring, flowers open only after relatively warm,

sunny days but not on cold and/or overcast afternoons or evenings. Between late August and the end of September, anthesis is crepuscular, occurring at dusk (± 20 min before sunset at 18:00). Later in the season, October to April, anthesis is nocturnal, taking place only after sunset. Flowers close well before sunrise at about 04:00. Most flowers last up to seven (eight in some individuals) days. There is variation between the timing of presentation between the male and female phases in the population. In some individuals there is almost complete overlap of pollen presentation and stigma receptivity, while in others dehiscence of the anthers starts one hour before the stigmas become receptive. On the first evening, at the onset of anthesis, the five stigmas are dry and clearly distinguishable and stigmatic papillae are clearly visible. Stigmatic secretion, anther dehiscence and nectar production start within one hour after the onset of anthesis.

A strong sweet odour is emitted by the flowers during the first four evenings (Figure 1) but becomes almost undetectable from the fifth night. Osmophores could not be detected when staining with neutral red. No odour could be perceived during daytime.

Nectar and pollen are available from the start of anthesis until day five. At the start of the second

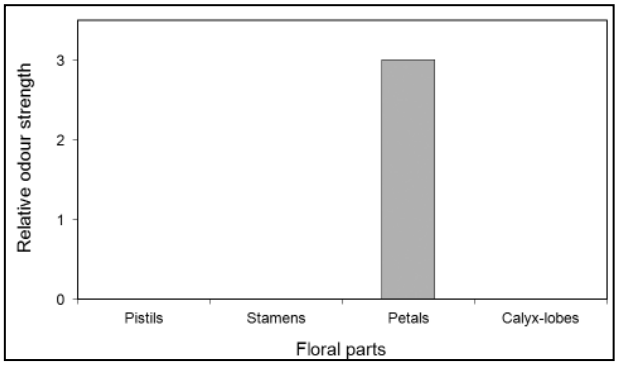

FIGURE 1.—Contribution to odour release by different floral parts of *Stomatium bolusiae*.

evening, the stigmas are already clogged and indistinguishable from each other as a result of prolific stigmatic secretion. Pollen is still available and the odour is still strong (Figure 2). At this stage the sticky pollen is available in large quantities and easily noticeable. On the fifth evening, odour and nectar production stop. At this stage noticeably fewer pollen grains are left in the anthers. At the end of night seven, the calyx lobes fail to fold back to their original position, indicating the end of anthesis. Depending on the longevity of a specific flower, the reproductive period appears to be between days one and five (occasionally six).

The average number of pollen grains is 274 314 per flower. The highest number of seeds observed in mature cross-pollinated capsules was 262. The pollen-ovule ratio of *S. bolusiae* is therefore estimated to be 1 047:1. Staining with Alexander's stain indicated that 94% of the pollen grains were potentially viable whereas the FCR test procedure indicated that only 75% of the pollen grains with live protoplast were germinable.

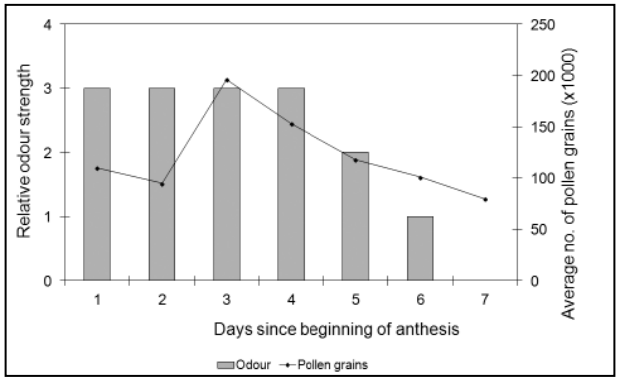

FIGURE 2.—Relative odour strength and availability of pollen grains during life span of flowers of *Stomatium bolusiae*. Shaded area, odour; graph, pollen grains.

Pollination experiments

Fruits were formed in 91% of the flowers that were marked to test for natural fruit set (open pollination) during September and October 1998/99. The index of seed set in these fruits was 58.4% and 75% in cross-pollinated flowers (xenogamy). In self-pollinated flowers, pollen grains germinated but pollen tube growth was arrested in the style and none of these flowers set seed (Figure 3A, B). None of the flowers that were tested for geitonogamy set seed.

Pollinator behaviour, attractants and rewards

Honeybees, *Apis mellifera* (Hymenoptera: Anthophoridae) visit the open flowers during early dusk. They approach the flowers from downwind and alight on top of the flowers. As the recess inside the flower is too small for the bees to get into, they force their heads into the flower to collect nectar and pollen, touching the petals and anthers. During this action, they come into contact with the pollen and stigmas. Once they have started collecting the available rewards they do not move around on the flower. Pollen was found on the heads, thorax and mouth parts of the bees. The only other visitors of significance are nocturnal moths (*Spodoptera* sp., Lepidoptera: Noctuidae), which visited the flowers throughout the flowering season. Pollen was found on the proboscises of these moths. As with honeybees, moths approach the flowers from downwind, land directly on top of an open flower and collect nectar by pushing their proboscis into the recess of the flower. They do not force their bodies into the flower. Visitation rates of bees (15/plant/hour) were higher than that of moths (0.8/plant/hour). No ants were observed visiting the flowers.

Fruit and seed set

All capsules marked during September 2001 and February 2002 matured. The most unsuccessful period for fruit set was during November and December 2001 when only 68% and 56% capsules ripened (Figure 4). The highest seed set was achieved during early summer (September and October 2001) and late autumn (May 2002). The highest number of seeds was produced during October 2001 (122 seeds per capsule, 58% capsules ripened). The lowest seed set was recorded during

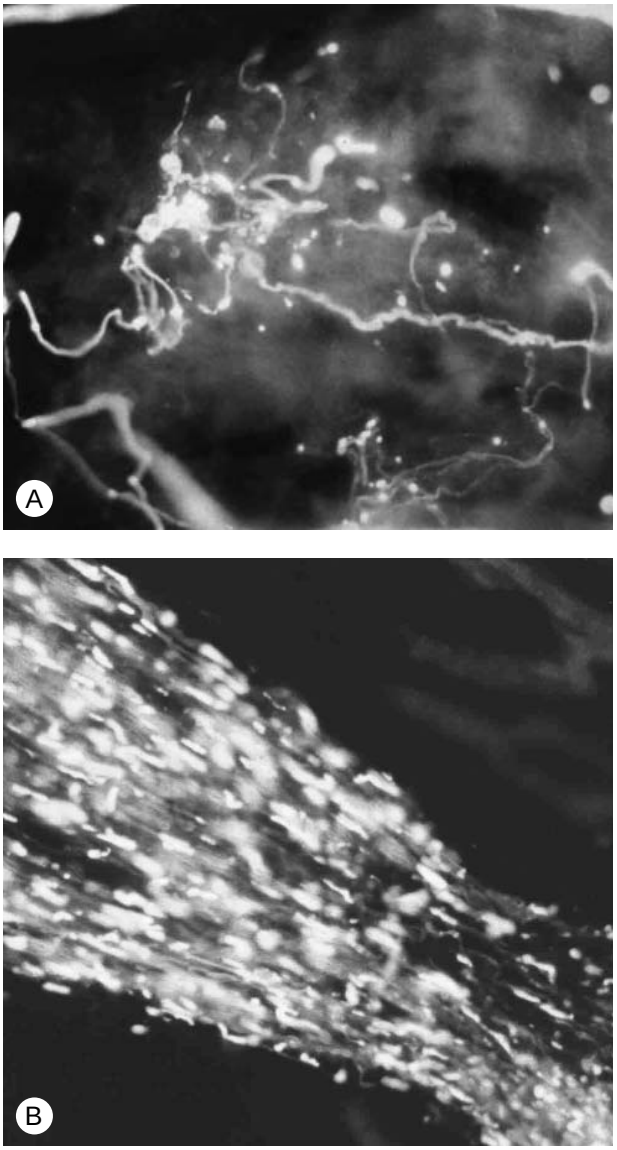

FIGURE 3.—Fluorescent micrograph of pollen tube growth in stigma and style of *Stomatium bolusiae* after staining with decolourised aniline blue: A, pollen tube growth of self-pollinated flowers arrested in style (× 72); B, normal growth in a cross-pollinated flower (× 18).

November and December 2001 and January 2002 when on average less than three seeds per capsule were produced. Flowering did not take place during midwinter (June/July) and no fruits or seeds were produced.

DISCUSSION

Stomatium bolusiae flowers are hermaphroditic and only bisexual flowers are borne. The life span of the flowers is seven days. In some flowers there is almost complete overlap of pollen presentation and stigma receptivity, as dehiscence of the anthers starts one hour before the stigmas become receptive in the majority of flowers. In others, this time lapse was not more than a few minutes and in some the male and female functions were not separated and overlapped completely. Furthermore, most individual plants have more than two receptive flowers, representing different days during its life span, at any one time. There is no clear separation (temporal or spatial) of male and female functions throughout anthesis. Flowers of *S. bolusiae* can therefore not be regarded as protandrous as described for most mellitophilous flowers by Hartmann (1991), therefore floral behaviour indicates homogamy. Furthermore, the wet receptive surfaces of the papillate stigmas as well as the arresting of pollen tube growth in the short style and not on the stigmas, suggest gametophytic self-incompatibility (Heslop-Harrison & Shivanna 1977).

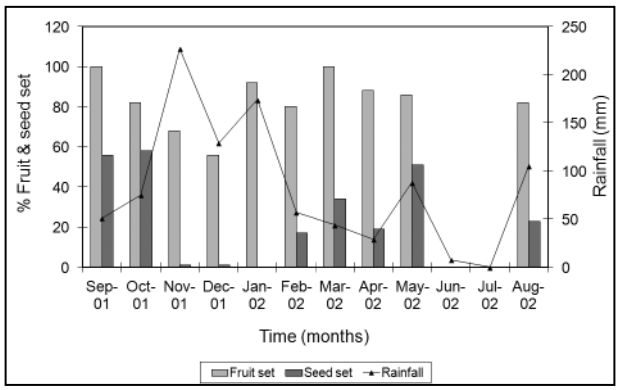

FIGURE 4.—Correlation of fruit and seed set of *Stomatium bolusiae* from September 2001 to August 2002, with rainfall received during same period. Pale shaded area, fruit set; dark shaded area, seed set; graph, rainfall.

Although the petals have been indicated as the source of the flower fragrance, the source could not be determined by staining. The P/O ratio as used by Cruden (1977) indicates that *S. bolusiae* is a facultative xenogamous species. Dafni (1992) regards this ratio as a reflection of the breeding system but cautions that each case should be studied in relation to its specific pollination syndrome. Dafni (1992) furthermore sets the standards for evaluating breeding systems based on the P/O ratio at the family level and not at the species level. Currently not enough data/information exists for the mesembs. More studies should thus be conducted before any such conclusions can be drawn relevant to the family. Results obtained from pollination experiments showed that none of the pollen tubes in self-pollinated flowers grew the length of the style and that none of the flowers, thus treated, set seed. This confirms self-incompatibility. Despite the simultaneous presentation of male and female functions and severe clogging of the stigmas by self-pollen, *S. bolusiae* should not be regarded as a facultative but as an *obligate* xenogamous species.

Seed set resulting from cross-pollinated flowers (75%), in comparison to natural seed set of 58.4%, indicates an effective pollination mechanism. The flowers are obviously not adapted for a specialist pollinator. The highly specific anthesis time excludes visits from all but two species, resulting in a very specific pollination mechanism. Flowers earlier in the season exhibit crepuscular anthesis, at which time they are pollinated by honeybees, a very common pollinator. Bees are part of a generalist pollination system, visiting a wide range of flowers and are thus polylectic. They are involved as the main pollen vectors in a number of pollination studies conducted in the Free State (Zietsman 1990, 1991, 1993, 1994, 1998). These studies all demonstrated the

sharp decline in pollinator visits in the late afternoon. Since no other plants were in flower in the proximity of the *Stomatium* population, the crepuscular activity of the bees during the early part of the daily opening of the flower is perhaps a result of food scarcity. The only visitors encountered during the night were noctuid moths. Nocturnal anthesis, anther dehiscence, nectar secretion and fragrance production in this species is consistent with adaptation for nocturnal moth pollination. Most of these features are also consistent with bee pollination. *S. bolusiae* must therefore be regarded as a melitto- and phalaenophilous species exhibiting a bimodal pollination system (Manning & Goldblatt 2005).

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REFERENCES

ALEXANDER, M.P. 1969. Differential staining of aborted and non-aborted pollen. *Stain Technology* 44: 117–122.

BAWA, K.S. 1974. Breeding system of tree species of a lowland tropical community. *Evolution* 28:85–92.

BITTRICH, V. & HARTMAN, H.E.K. 1988. The Aizoaceae—a new approach. *Botanical Journal of the Linnaean Society* 97: 239–254.

CHESSELET, P., BURGOYNE, P., KLAK, C., KURTZWEIL, H., DOLD, A.P., GRIFFON, N.J. & SMITH, G.F. 2003. Mesembryanthemaceae. In G. Germishuizen & N.L. Meyer (eds), Plants of southern Africa: an annotated checklist. *Strelitzia* 14: 633–738. National Botanical Institute. Pretoria.CRUDEN, R.W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.

DAFNI, A. 1992. *Pollination ecology, a practical approach*. Oxford University Press. Oxford.

GESS, S.K. & GESS, F.W. 1989. Flower visiting masarid wasps in southern Africa (Hymenoptera: Vespoidea: Masaridae). *Annals of the Cape Provincial Museums. Natural History* 18: 95–134.

GUNATILLEKE, C.V.S. & GUNATILLEKE, I.A.U.N. 1984. Some observations on the reproductive biology of three species of *Cornus* (Cornaceae). *Journal of the Arnold Arboretum*. 65: 419–427.

HAMMER, S.A. 2002. Dumpling and his wife: new views of the genus *Conophytum*. EAE Creative Colour Ltd. Norwich.

HARTMANN, H.E.K. 1978. Monographie der Gattung *Argyroderma* N.E.Br. (Mesembryanthemaceae Fenzl). *Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg* 15:121–235.

HARTMANN, H.E.K. 1991. Mesembryanthema. *Contributions from the Bolus Herbarium*. 13. Bolus Herbarium, Cape Town.

HARTMANN, H.E.K. (ed.) 2002a. *Illustrated Handbook of Succulent Plants: Aizoaceae A–E*. Springer-Verlag. Berlin.

HARTMANN, H.E.K. (ed.) 2002b. *Illustrated Handbook of Succulent Plants: Aizoaceae F–Z.* Springer-Verlag. Berlin.

HARTMANN, H.E.K. & DEHN, M. 1987. Monographien der Subtribus Leipoldtiinae. VII. Monographie der Gattung *Cheiridopsis* (Mesembryanthemaceae). *Botanische Jahrbücher* 108: 567–633.

HARVEY, C.F. & BRAGGINS, J.E. 1985. Reproduction in the New Zealand taxa of *Pomaderris* Labill. (Rhamnaceae). *New Zealand Journal of Botany* 23: 151–156.

HESLOP-HARRISSON, J., HESLOP-HARRISSON, Y. & SHIVANNA, K.R. 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical and Applied Genetics* 67: 367–375.

HESLOP-HARRISSON, Y. & SHIVANNA, K.R. 1977. The receptive surface of the angiosperm stigma. *Annals of Botany* 41: 1233–1258.

JEFFERIES, C.J. & BRAIN, P. 1984. A mathematical model of pollen-tube penetration in apple styles. *Planta* 160: 52–58.

JUERGENS, A. 2004. Nectar sugar composition and floral scent compounds of diurnal and nocturnal *Conophytum* species (Aizoaceae). *South African Journal of Botany* 70,2: 191–205.

LIEDE, S. & HAMMER, S. 1990. Aspects of floral structure and phenology in the genus *Conophytum* (Mesembryanthemaceae). *Plant Systematics & Evolution* 172: 229–240.

LIEDE, S., HAMMER, S. & WHITEHEAD, V. 1991. Observations on pollination and hybridization in the genus *Conophytum* (Mesembryanthemaceae). *Bradleya* 9: 93–99.

MANNING, J.C. & GOLDBLATT, P. 2005. Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *International Journal of Plant Science* 166: 459–474.

MARTIN, F.W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain technology* 34: 125–128.

MUCINA, L. & RUTHERFORD, M.C. (eds). 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia* 19. South African National Biodiversity Institute, Pretoria.

PETER, C.I., DOLD, A.P., BARKER, N.P. & RIPLEY, B.S. 2004. Pollination biology of *Bergeranthus multiceps* (Aizoaceae) with preliminary observations of repeated flower opening and closure. *South African Journal of Science* 100: 624–629.

SAMAHA, R.R., BOYLE, T.H. & MULCAHY, D.L. 1989. Self-incompatibility of *Zinnia angustifolia* HBK (Compositae). I. Application of visible light and florescence microscopy for assessment of self-incompatibility. *Sexual Plant Reproduction* 2: 18–23.

SMITH, G.F., CHESSELET, P., VAN JAARSVELD, E.J., HARTMANN, H.E.K., HAMMER, S., VAN WYK, B., BURGOYNE, P., KLAK, C. & KURZWEIL, H. 1998. *Mesembs of the World*. Briza Publications, Pretoria.

STRUCK, M. 1994. Flowers and their insect visitors in the arid winter rainfall region of southern Africa: Observations on permanent plots. Composition of the anthophilous insect fauna. *Journal of Arid Environments* 28,1: 45–50.

STRUCK, M. 1995. Land of blooming pebbles: flowers and their pollinators in the Knersvlakte. *Aloe* 32,3&4: 56–61.

THIEDE, J., OPEL, M.R. & HAMMER, S.A. 2011. Flower pigment patterns and systematics of *Conophytum* N.E.Br. (Aizoaceae). *Haseltonia* 16: 9–15.

VAN WYK, A.E. & LOWREY, T.K. 1988. Studies on the reproductive biology of *Eugenia* L. (Myrtaceae) in southern Africa. *Monographs in Systematic Botany* 25: 279–293.

VOGEL, S. 1954. Blütenbiologische Typen als Elemente der Sippengliederung. In: W. Troll & H. von Guttenberg, *Botanische Studien 1*. Fisher, Jena.

WASER, N.M. & PRICE, M.V. 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interactions. In C.E. Jones & R.J. Little, *Handbook of experimental pollination biology*. Scientific and Academic Editions, New York.

ZIETSMAN, P.C. 1990. Pollination of *Z. mucronata* subsp. *mucronata* (Rhamnaceae). *South African Journal of Botany* 56: 350–355.

ZIETSMAN, P.C. 1991. Pollination biology of *Grewia occidentalis* (Tiliaceae). *South African Journal of Botany* 57: 348–351.

ZIETSMAN, P.C. 1993. Pollination biology of *Pelargonium dolomiticum* Knuth (Geraniaceae). *South African Journal of Botany* 59: 259–264.

ZIETSMAN, P.C. 1994. Autogamy in *Sesamum triphyllum* Welw. ex Ashers. var. *triphyllum* (Pedaliaceae). *Navorsinge van die Nasionale Museum Bloemfontein.* 10: 633–646.

ZIETSMAN, P.C. 1998. Pollination biology of *Cotyledon orbiculata* L. var. *dactylopsis* Toelken (Crassulaceae). *Navorsinge van die Nasionale Museum Bloemfontein* 14: 81–96.