Floral biology of Zantedeschia aethiopica (L.) Spreng. (Araceae)

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Floral morphology, stages in floral development and insect visitation in Z. aethiopica are reported. Floral development can be divided into five phases: pre-female, female, male, fruit development and fruit ripening. Plants are self-incompatible and separation of sexes is achieved by marked protogyne. Z. aethiopica is the only member of the genus in which the basal part of the spathe turns green during fruit maturation while the apical part withers away. This species is further unique in that the berries turn orange, soft and mucilaginous on ripening. Both Z. aethiopica and Z. odorata have staminodes present amongst the ovaries and a faint scent is evident. Several insects, especially beetles, were seen visiting the inflorescence. Observations on Z. aethiopica are compared with other members of the genus, notably Z. albomaculata subsp. albomaculata.

Keywords: Araceae, beetle pollination, flower morphology, pollen, Zantedeschia aethiopica.

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Introduction

The genus Zantedeschia Spreng. is endemic to southern Africa and comprises seven species: Z. aethiopica (L.) Spreng., Z. albomaculata (Hook.) Baill., Z. rehmannii Engl. Z. penitandii (Watson) Wittm., Z. elliotiana (Watson) Engl., Z. jucunda Letty and Z. odorata P.L.Perry. Z. albomaculata has three subspecies, the typical one, as well as Z. albomaculata subsp. macrocarpa (Engl.) Letty and Z. albomaculata subsp. valida Letty. Letty (1973) grouped the species into two informal sections, namely the typical section containing only one species, Z. aethiopica, and a section containing the remaining species. According to her, Z. aethiopica differs from the latter group in that the plants do not die down in winter, female flowers are intercalated with staminodes, peduncles remain upright during fruiting, and fruits turn orange, soften and become mucilaginous on ripening. This species is also the only one in which the upper portion of the spathe eventually withers and turns brown, and the lower portion turns green during fruiting. The recently described Z. odorata (Perry 1989) displays characters of both sections.

Apart from a few casual observations reported by Marloth (1915) and Jackson (1986), no detailed study on the floral biology and pollination of Zantedeschia has yet been undertaken. Furthermore, although pollinators have been recorded for many araceous genera (Grayum 1986), those of the southern African aroids have received no attention.

Z. aethiopica is perhaps the best known member of the genus. It is a popular horticultural subject with many cultivars that are grown all over the world. This species was chosen for the study because it is common and readily available. Where possible, observations were also made on Z. albomaculata subsp. albomaculata for comparison. The objectives of this study were to describe the various stages in floral development, to suggest possible sites of fragrance emission, to consider the role of scent in attracting insects and to assess the possible role of different insect visitors.

Materials and Methods

Field observations

Field observations on natural populations were made at three sites in Natal: Nottingham Road (2929BD), Cato Ridge (2930DA) and Merebank (2930DD). Sites were visited during the main flowering period (August to January) in 1992/3, 1993/4 and 1994/5. Activity of various insect visitors was observed. Additional observations were made on plants during collecting trips in Mpumalanga and the Western Cape Province.

Morphology of reproductive structures

Spathe was collected from different populations throughout South Africa and preserved in formalin-acetic acid-alcohol (FAA) at a ratio of 1:1:18. Spadices and flowers were examined using a Wild stereo microscope. An assessment of stamens and pistils was undertaken for 13 inflorescences from a single population at Nottingham Road. Dried, unacetolysed pollen grains were studied using a scanning electron microscope (SEM), following standard procedures.

Stages of floral development

Plants collected from the wild were cultivated in a greenhouse. Different stages of floral development observed in these plants were photographed under a Wild Autophotomat MPS 55 photomicroscope. Inflorescences of these plants were also used to record the maturation of sexes and development of fruit.

Breeding behaviour

In the greenhouse, inflorescences of Z. aethiopica and Z. albomaculata subsp. albomaculata were bagged to test for self-incompatibility. Fine mesh nylon bags of 150 × 200 mm were placed over the inflorescences on emergence.

Collecting of insects

Spathe containing insects were collected in the field and stored in honey jars. In the laboratory, insects were killed and preserved by freezing. This method ensured that pollen remained on the bodies of the insects. The outer surface of insect bodies was examined for the presence of pollen, under a dissecting microscope. Pollen grains of members of the genus are white and are therefore easily visible on the usually dark bodies of the insects. In addition, insects were randomly collected from different inflorescences in a population. Insects were identified at the Durban Natural Science Museum.

Determination of scent

Using fresh material, the spathe, male and female portions of the inflorescence were separated and placed individually in glass bottles which were sealed. After 10 and 30 min, three persons not familiar
ovary is surrounded by three pathulate staminodes (Figure 1). The morphology of the reproductive structures of *Zantedeschia* and *Z.

Table 1  Ratio of ovaries to anthers per spadix in *Z. aethiopica*

<table>
<thead>
<tr>
<th>Inflorescence</th>
<th>Number of ovaries</th>
<th>Number of anthers</th>
<th>Ovaries: anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>1392</td>
<td>1:22</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>1242</td>
<td>1:15</td>
</tr>
<tr>
<td>3</td>
<td>94</td>
<td>928</td>
<td>1:10</td>
</tr>
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<td>4</td>
<td>70</td>
<td>1200</td>
<td>1:17</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>1062</td>
<td>1:18</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
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<tr>
<td>15</td>
<td>97</td>
<td>1495</td>
<td>1:15</td>
</tr>
<tr>
<td>Mean</td>
<td>72.40</td>
<td>1088.73</td>
<td>1:15</td>
</tr>
</tbody>
</table>

with scent in this plant were asked to smell each bottle and to record the presence of a scent. To locate presumptive osmophoric tissue, freshly collected whole spathe were stained in a 1:1000 neutral red-water solution, following the procedure used by Stern et al. (1986), except that the staining period was extended to one hour, rather than 20 min. After a rinse in tap water, the spathes and spadix were carefully examined for any possible floral patterns. In addition to the neutral red test, longitudinal sections of anthers and staminodes were examined under oil immersion with an Olympus compound microscope, to determine the presence of osmophores.

Results and Discussion

Morphology of reproductive structures

As is characteristic of the family Araceae, flowers of *Zantedeschia* are arranged on a fleshy axis (spadix) enclosed in a large, leaf-like bract, the spathe. The upper portion of the spadix is covered by a densely crowded mass of stamens, while the female flowers form a well-developed zone at the base. Male flowers are made up of one to three sessile anthers. Since it was not possible to resolve the exact number of stamens per male flower, the total number of anthers on the spadix was counted. Table 1 indicates the numbers of ovaries and anthers for 15 inflorescences.

Anthers range from about 400 to almost 1 500 and the ovaries from about 40 to almost 100 per spadix. From Table 1 it is clear that the ratio of anthers to ovaries is very variable. On average, the anthers are about 15 times more than the ovaries. The anthers are free and each is characterized by two terminal pores. Ovaries are ovoid in shape with a truncate stigma and 3-locular with 1-4 ovules per locule. The morphology of the reproductive structures is rather similar for all species, except that in *Z. aethiopica* and *Z. odorata*, staminodes are interspersed amongst the ovaries. Each ovary is surrounded by three spathulate staminodes (Figure 1). Although apparently without function, their presence is taxonomically useful in separating these two species from the remaining members of the genus.

Flowering behaviour

The development of flowers can be divided into the following five sequential phases (Figure 2a-e):

Pre-female phase. Spathe is tightly rolled. This stage lasts for about three days from the day the emerging spathe becomes visible. The stigmas are non-receptive and the anthers immature.

Female phase. Spathe begins to unwind. This phase, which lasts for about six days, can be discerned by well-developed glistening stigmatic areas, indicating their receptivity (Figure 3, arrows). A faint scent is emitted from the inflorescence. The flowers are markedly protogynous, with anthers still immature.

Male phase. Spathe is fully opened. All the anthers on the spadix dehisce simultaneously (Figure 4). Anthesis lasts for about 10 days. A faint scent is still present at this stage. The stigmatic surface degenerates, signified by a browning of the stigmas (Figure 5, arrow).

Fruit development. During this period of about 30 days the berries enlarge to reach a size of about 13 × 10 mm. By this stage, the staminodes have shrivelled and are squashed among the expanding berries. As the berries mature, the infructescence expands vertically and laterally. The upper portion of the spathe begins to wither, whereas the lower portion turns green (Figure 2d).

Fruit ripening. The fruit ripen sequentially from apex to base (Figure 2e). When all fruits are fully ripe, the infructescence is bright orange.

Breeding behaviour

Bagging experiments indicated that spontaneous selfing does not occur in *Z. aethiopica*, unlike in *Z. albomaculata* subsp. *albomaculata*, where selfing resulted in seed set. Solitary bagged inflorescences of *Z. aethiopica* simply withered away, while those of *Z. albomaculata* produced fruit. *Z. aethiopica* clearly requires cross-pollination for seed set. It is unfortunate that selfing could not be tested for the remaining species as these failed to flower under greenhouse conditions.

Pollen morphology and extrusion

Pollen exine sculpturing is psilate in *Zantedeschia*. Grayum (1986) found a positive correlation between psilate pollen and beetle pollination in the Araceae. He further suggests that pollen is attached onto the smooth hard-bodied insects such as beetles through the agency of sticky secretions of the stigma or of the
inner spathe surface as in Dieffenbachia Schott. In Zantedeschia, pollen grains are extruded through the anther pores in fine long threads (Figure 4, arrows), held together by a sticky substance. Whether pollen is also held together electrostatically is unknown. Presumably, the release of pollen in threads requires only one end of the thread to attach itself to the insect body for the entire thread to be transported. Under field conditions, these strings of pollen grains rarely accumulate, as they are soon removed by insects. However, under greenhouse conditions, pollen grains accumulate on the spadix, eventually falling to the base of the spathe. In Z. albomaculata subsp. albomaculata, in the absence of vectors, pollen has been observed to fall in large masses onto the stigmas.

The mechanism for the release of pollen in flowering plants is achieved by shrinkage of the endothecial layer as the cells lose water (Fahn 1974). In general, the inner pericinal walls of the

Figures 3-5 3. Female portion of spadix during the female phase in Z. aethiopica (spathe removed). Female flowers interspersed with stamnodes. Note the glistening stigmas. Scale bar = 3 mm. 4. Male portion of spadix during the male phase in Z. aethiopica (spathe removed). All anthers have dehisced simultaneously, releasing the white pollen in fine threads (arrow). Scale bar = 1.5 mm. 5. Female portion of the spadix during the male phase, showing the browning of the stigmatic surfaces in Z. aethiopica (spathe removed). Scale bar = 2 mm.
endothelial cells or fibrous layer are thickened and this forces the anther slits to open during dehydration (Fahn 1974). Squeezing of pollen grains through pores in Z. aethiopica and other pericentral members of the Araceae is due to the thickening being on the outer rather than the inner pericentral walls of the endothelial cells (Weberling 1989). The inverse position of the thickening probably creates an inward pressure during water loss, thereby compressing the pollen sac, which releases the pollen in threads through the pore.

A range of insects was collected from the inflorescences of Z. aethiopica, including mainly beetles of the genera Anoplochilus MacLeay and Leucocelis Burmeister (Family Scarabaeidae). The bodies of all insects were covered with pollen. The species therefore, does not appear to have a single specific pollinator. Pollen mass has been associated with beetle pollination (Grayum 1986) and since beetles were the predominant group of insects collected from spathes, Z. aethiopica is considered to be beetle pollinated. The beetles appear to enter the spathes at the female stage, and remain there until after anthesis, clustered mainly around the female portion of the spadix. It could not be confirmed whether the beetles were feeding on the stigmatic fluid. The regular presence of mating pairs indicates that the base of the spathes provides a suitable niche for mating. Observations suggest that arachnids and other insects (e.g. bees and flies) merely seek shelter or trap their food in the spathes, thereby accidently effecting pollination.

Scent production

Marloth (1915) recorded the presence of scent in the spathes of Z. aethiopica. Perry (1989), on the other hand, reported its absence in Z. aethiopica, but its presence in Z. odorata, hence the choice of specific epithet. Vogel (1990) expressed the opinion that there is no discernible smell in the inflorescences of Zantedeschia, and if present, it serves no function. This study confirmed that the inflorescence of Z. aethiopica produces a faint scent which can easily be missed if not captured at the correct floral stage. All three persons asked to verify which part of the inflorescence produced an odour indicated the presence of a scent in the female zone, which suggests that either the staminodes or pistils are sites for osmophores.

Osmophores are common in the Araceae. They have been reported in the male flowers (Philodendron Schott), the flowers (Spatiphyllum Schott), the spathe (Cryptocoryne Fisch. ex Wyll.) and the appendix of the spadix (Amorphophallus Blume ex Decne.) (Vogel 1990). Staminodes and fertile anthers in Homalomena cordata (Houtt.) Schott, a member of the same subfamily as Zantedeschia, were found to have the histological composition of osmophores (Eyre et al. 1967). Although, tissues of the spathe and spadix did not stain with neutral red, longitudinal sections through the anther (Figure 6a) and staminode (Figure 6b) of Z. aethiopica indicated a structure similar to that in H. cordata. Since scent in Zantedeschia is restricted to stamino-bearing species, it appears likely that the staminodes are responsible for scent emission. However, similarity in the histological composition of anthers and staminodes, as well as in anthers of all species, reduces the possibility of staminodes being involved in scent production. A survey of the histological composition of pistils in the scented and non-scented species would provide evidence as to whether the pistils are responsible for scent production. The question that arises is by what means are insects attracted to spathes of the non-scent producing species of Zantedeschia? Should the latter species prove to be self-compatible, then this state and the lack of detectable scent may be correlated.

Gottsberger (1977) reported that beetles visiting flowers may feed on pollen, nectar or flower organs like petals, tepals, stamens and carpels, and thereby cause considerable damage to flowers. In the scented species of Zantedeschia, staminodes may also provide protection against herbivores. The lateral walls of anthers and staminodes were found to be lined with several tannin cells. The ovaries however, lack tannin. Secondary substances such as tannins have evolved in response to natural selection for defence against herbivores and insects (Whittaker & Feeny 1971). Therefore, it may be assumed that tannins in staminodes of Z. aethiopica and Z. odorata act as antifeedants to beetles, thereby protecting the ovaries. The base of the spathe is cool and moist and therefore an ideal environment for fungal growth. Whittaker & Feeny (1971) have quoted several publications where it was found that tannins also inhibit fungal growth. Hence, tannins in the floral structures of Zantedeschia may protect the plants against animals and fungi.

Spathe greening

Once the ovules have been fertilized, the white spathe begins to turn green. Greening of the spathe occurs in all species of Zantedeschia. However, in Z. aethiopica only the lower portion of the spathe turns green, whereas the upper portion withers away to display the orange berries to dispersal agents. Fruit exposure is not necessary in the remaining species as seed dispersal is achieved by mere disintegration of the infructescence. Hence in these species the entire spathe turns green. Spathe greening is induced by endogenous cytokinins produced by the fruit and translocated to the spathe (Chaves das Neves & Pais 1980).

Figure 6 Papillate epidermal cells as in Homalomena cordata. a. L.S. anther. Scale bar = 12 μm. b. L.S. staminode. under oil immersion.
These authors have further identified the cytokinin 6-(o-hydrobenzylamino)-9-B-D-ribofuranosylpurines as the spathe-greening factor in Z. aethiopica.

Fruits
The orange coloration of the ripe berries, softening of the pericarp, erect peduncle and the withering away of the upper portion of the spathe to expose the berries in Z. aethiopica all play a role in attracting dispersers. In this study no continuous field observations were conducted to detect the seed-dispersal agent (if any) of this species. However, casual observations suggest that the berries are eaten by birds. To confirm this, and to determine which bird species feed on the berries, more extended field observations are required.

Conclusions
The present results provide essential information on the breeding behaviour of Z. aethiopica. The data correlates beetles with pollination, staminodes with possible scent production and/or defence, fragrance emission with self-incompatibility and berry phenology with bird dispersal. In addition, these observations provide characters useful for separating species and contribute to a better understanding of relationships within Zantedeschia.

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References