

A PRELIMINARY STUDY OF THE DISTRIBUTION OF POLLEN AND  
MICROGAMETOPHYTES ON STIGMAS OF A WIND POLLINATED SPECIES,  
STABEROHA BANKSII (RESTIONACEAE).

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DIGITISED

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## ABSTRACT

A detailed examination was made of the number of pollen grains and microgametophytes occurring on stigmas of a dioecious, wind pollinated species, Staberoha banksii. Stigmas were sampled from thirteen plants in a population at Cape Point. Patterns of variation among pistils in the size of the pollen load are related to factors influencing the efficacy of pollen capture and the availability of pollen. Within-plant variation is affected by the position of the spikelet on the plant, position of the flower on the spikelet and the age of the stigma. Between-plant variation is affected by pollen availability as determined by the proximity and density of the pollen source. Distance to the nearest male plant explained 45% of the variation in size of the pollen load. The numbers of pollen grains per pistil varied widely, ranging from 0 to <sup>over</sup>100. The mean number per plant varied from 4.36 to 28.04. On average there were 5.19 microgametophytes per ovule, the numbers ranging from 0 to 45 per pistil. 66% of the stigmas had two or more microgametophytes, indicating that there is considerable potential for gametophytic competition. Seedset was very high (greater than 90%) and showed no decline in female plants isolated 7 to 13 meters from the nearest pollen source. Although pollen load size was shown to be limited by increased distances, it is concluded that the quantity of pollen is not limiting fertilization in this population. The results of this preliminary study should be beneficial for future studies concerning the efficiency of wind pollination in the Restionaceae.

## INTRODUCTION

Wind pollination is generally considered to be an inefficient, wasteful process relying on the excessive production of pollen (Faegri & Van der Pijl 1979). Recent work by Niklas (1984, 1985) however has shown that some wind pollinated plants generate specific air currents around their reproductive organs which enhance the efficiency of pollination. Studies on Pinus and Ephedra indicate that these plants selectively capture conspecific pollen and that ovulate organs act as passive filtration devices (Niklas & Paw 1983, Buchmann et al. 1989).

The efficiency of wind pollination is generally assumed to decrease rapidly with increasing distances between conspecifics (Regal 1982, Whitehead 1983). This observation is based on Sutton's formula which predicts that the concentration of airborne pollen decreases at a rate which is inversely proportional to the square of the distance travelled (Tauber 1965, as cited in Levin & Kerster 1974). Several studies have shown the leptokurtic dispersion of windborne pollen but evidence from natural populations is limited and indirect (eg. Player 1979, Buchmann et al. 1989).

The effect of distance on pollination efficiency is typically inferred from measurements of airborne pollen density at various distances from a pollen source (eg. an isolated plant). This method may underestimate the efficiency of wind pollination for several reasons. First the use of pollen traps such as rotorod or burkard samplers ignores the efficiency of the stigma as a filtering device. Yet there is growing evidence that pollen capture is influenced by the size, shape and orientation of the collecting object. It has been shown that foliage leaves and reproductive structures such as bracts and sepals may increase the probability of pollen deposition by deflecting

air currents toward the stigma (Niklas 1985, Niklas & Buchmann 1985, 1987). Second, pollen emitted from a point source behaves very differently from that of diffuse origin (Levin & Kerster 1974). Measurements made from an isolated plant therefore emphasize the leptokurtic nature of pollen dispersal and do not necessarily reflect the distribution of pollen in a population. An alternative approach to investigate the efficiency of wind pollination would be to determine how much airborne pollen is entrained by the stigmas themselves and relate this to the density and proximity of the pollen source (i.e. conspecific individuals). I am unaware of any studies which have examined the distribution of pollen on stigmas in natural populations and related this to pollen availability.

Berry & Calvo (1989) showed that seedset was strongly dependent on flowering density in two anemophilous species of Espeletia. There are problems however associated with the use of seedset to estimate pollination efficiency as seedset is a function of both pollen and resource availability. In Taxus canadensis for example, plant spacing was inversely related to pollination success (proportion of ovules which had been pollinated) but only weakly correlated to seedset (Allison 1990). Although it provides a minimum estimate of pollen dispersal, seedset gives no indication of the extent of pollen transfer between plants, and whether the process is strongly distance limited.

It is clear from the above discussion that to assess the efficiency of anemophily as a pollination mechanism, we need more direct measurements of the amount of airborne pollen landing on stigmas and the distances over which it is dispersed. In this study I report on the number of pollen grains occurring on stigmas sampled from a population of Staberoha banksii <sup>anthur</sup> (Restionaceae) and attempt to relate this to the availability of pollen within the population. The

Restionaceae forms an important element of South African fynbos vegetation, as the family is both ecologically abundant and taxonomically diverse. 314 species occur in the Cape Floristic Kingdom. They are dioecious, wind pollinated and typically occur in mixed stands of several species.

Several aspects of its biology make the Restionaceae a particularly interesting family for studies concerning the efficiency of wind pollination. Although some species occur in dense monospecific stands, plants often occur as isolated individuals amongst other vegetation, and male and female tussocks may therefore be quite widely spaced. Nothing is known of the extent of pollen transfer within and between populations, or how reproductive isolation is maintained between species. Unanswered questions concerning the efficacy of wind pollination include: What fraction of the flowers in a population receive pollen, how much lands on a stigma and how does this relate to distance from the pollen source? What proportion of the pollen grains germinate, and to what extent does competition occur between microgametophytes? Is there any evidence for the selective capture of pollen? Answers to these questions would make an enormous contribution to existing ideas on anemophily and the potential for gene flow by wind-dispersed pollen.

There is considerable variation in stigma morphology which may relate to different mechanisms of pollen capture (Linder, in press). In some genera the stigma is feathery and exserted (features typically associated with wind pollinated flowers), but in many cases flowers are hidden behind large bracts and hence are not directly exposed to the airstream. An investigation of the the functional significance of these bracts may provide insight into the mechanism of pollen capture and deposition in this group and shed light on how reproductive isolation is maintained in congeneric, sympatric species.

Intensive work on the systematics of the Restionaceae during the last decade has resulted in a well corroborated phylogeny (Linder 1984, 1985), but its reproductive biology has hardly been studied. Basic information concerning flowering phenology, pollination and seed dispersal is wanting (Linder, in press). Unfortunately flowering times cannot be obtained from herbarium records as most specimens were collected while in fruit.

I report here on a preliminary study of wind pollination in S. banksii. A detailed examination was made of the distribution of pollen grains and microgametophytes (germinating grains with pollen tubes attached) on stigmas of thirteen plants occurring in a population at Cape Point. The purpose of the study was to gain an understanding of the patterns of variation in the size of the pollen load and numbers of microgametophytes in a natural population, and to relate these to possible causes. Variation within and between plants is interpreted in terms of differences in pollen availability and the efficacy of pollen capture. The use of a single population as the sampling universe limits the conclusions which can be made from this study. Further work in different populations throughout its distribution would enable us to interpret pollen dispersal distances in terms of potential gene flow within and between populations.

## MATERIALS AND METHODS

### Study species

Staberoha banksii Pillans has a widespread distribution, ranging from the Cape Peninsula to Bredasdorp, and to the Hex River Mountains (Linder, 1985). Tussocks are 40 to 80cm tall and commence flowering in early winter (April/May). Male plants can be easily distinguished by their pendulous spikelets which are borne on simple culms. Female spikelets are spindle-shaped, erect, 2.5- 5cm long, and typically consist of 10 to 20 flowers arranged spirally on the floral axis. Flowering commences at the base of the spikelet and continues sequentially to the top (i.e. acropetally). The flower is sessile and situated at the base of a large bract 12 - 18mm long. The gynaecium is unilocular with a single ovule and 2 or 3 (rarely 4) flattened styles. The styles are sickle-shaped, about 5mm long and feathery on the inner margin. As the gynaecium is much shorter than the subtending bract, the stigma is not directly exposed to the atmosphere. This contrasts sharply with other members of the family (eg. Hypodiscus, Chondropetalum), where the stigma is exerted and hence directly exposed to pollen-bearing air currents. The fruit is an indehiscent nut which is wind dispersed. FIG. 1A.

*C. van der Merwe (R)*

### Study site and sampling procedure

Material was collected from a population of S. banksii in the Cape of Good Hope Nature Reserve (34°15'S, 18°25'E), approximately 35km south of Cape Town. The region has a mediterranean-type climate and receives a mean annual rainfall of 500mm - 600mm. The Cape Peninsula is exceptionally windy and gale force winds may reach 102km per hour. South easters of 16 to 40km per hour occur regularly during summer. Equally strong north westers, but usually of shorter duration, blow during winter (Tinley, 1985).

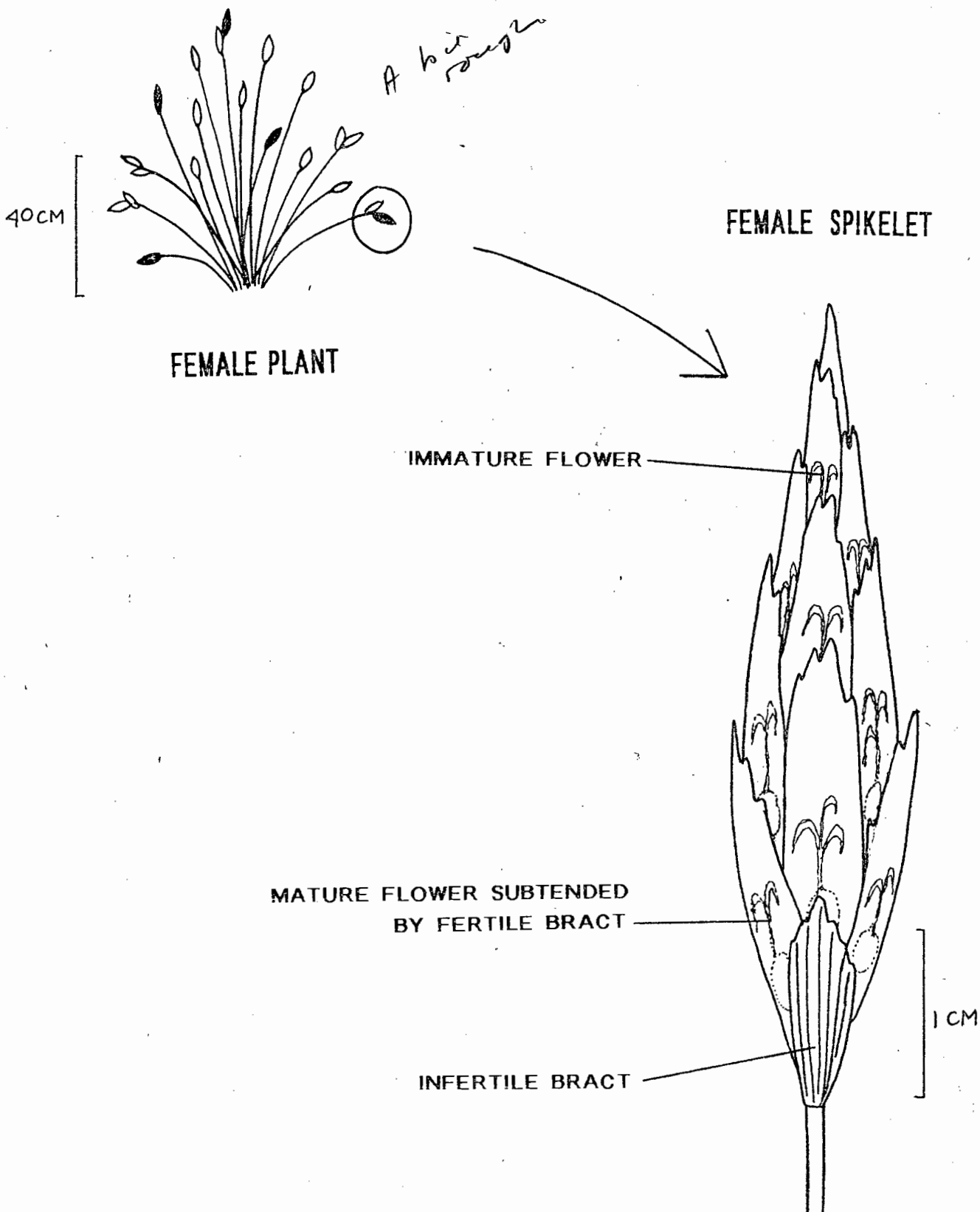


FIGURE 1A: General morphology of *Staberoha banksii* and structure of the female spikelet. Flowers (indicated by dotted lines) are situated behind each subtending bract.

Staberoha banksii is conspicuous on dry hillsides in the reserve (Taylor, 1969). It occurs in fairly dense stands, the average distance between plants being less than a metre. Populations are male-biased, the ratio of male:female plants being approximately 3:2. Plants are 40 - 80cm tall and range from large tussocks over 50cm in diameter to those consisting of only a few culms (5cm base diameter). Male and female tussocks are of similar height. S.banksii emerges from the surrounding vegetation by 5 to 40cm and is the only ~~restio~~ <sup>restio</sup> in the area which flowers during May.

*restio*

Material was collected on 1 May 1990 and fixed in 40% formalin: 80% alcohol: acetic acid (1:8:1 by volume). Five inflorescences (spikelets) were sampled from each of thirteen randomly chosen female plants. Spikelets were collected from different parts of the tussock to avoid any directional effect of the pollen source. The distance from each female to the nearest four male plants was measured to give an indication of the density and proximity of the pollen source.

The same population was sampled on 22 July 1990 to check seedset. 10 spikelets were collected from each of 20 female plants and the distance to the nearest four male plants was measured. Females were chosen so that they could be divided into two categories ("near" and "far") according to the proximity of the pollen source. Plants were termed "near" when the four nearest male plants occurred within a metre from the female and "far" when the nearest male plant occurred at a distance greater than 2.5m.

The following questions were addressed:

1. What determines the size of the pollen load on a pistil?
  - a. Age of the pistil
  - b. Pistil morphology (whether the pistil has 2 or 3 styles)

- c. Position of the pistil within the spikelet
  - d. Position of the spikelet on the plant
  - e. Distance to the nearest male plant
2. What is the size of the microgametophyte population and to what extent does competition exist between microgametophytes?

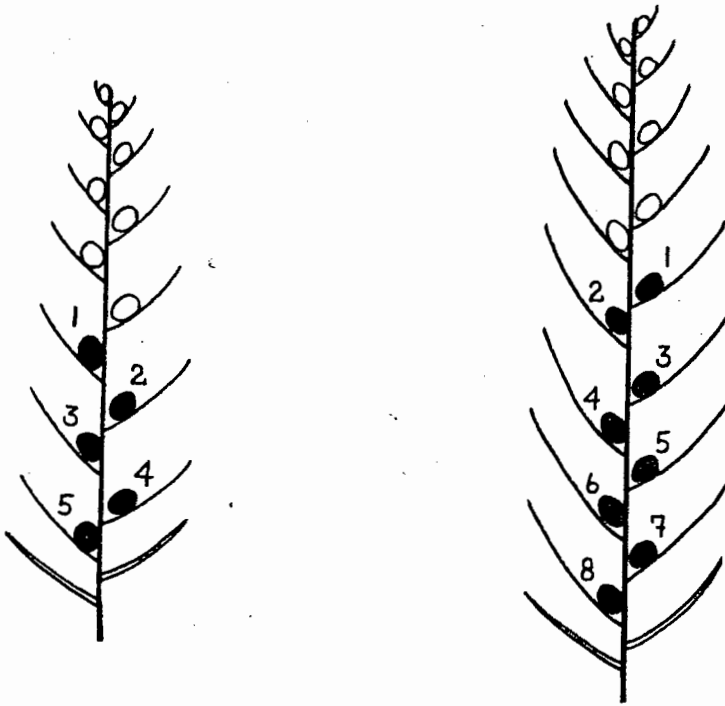
These questions were addressed by a detailed examination of the spatial distribution of pollen grains and germinating pollen tubes on thirteen plants.

#### **Pollen grain and pollen tube counts**

Pollen grains and microgametophytes were observed using the fluorescent staining technique described by Martin (1959). Stigmas were softened in a strong sodium hydroxide solution ( $\pm$  8M NaOH) for 12 to 24 hours to clear the tissue and permit adequate penetration of the dye. Next they were washed in water for 1 hour and stained for 3 hours in a 0.1% solution of water-soluble aniline blue dye dissolved in 0.1 M  $K_3PO_4$ . Stigmas were mounted on slides in a few drops of the fluorescent stain and observed without delay under a microscope. The stain was made up a day in advance and could be used for several days if stored in the dark. However material which had been left in it over the weekend (Friday 20h00 to Monday 13h00) fluoresced too faintly to see.

Slides were observed under UV light (365nm). Pollen grains stained bright yellow and were counted at 100x magnification. Microgametophytes were visualised by the yellow or pale blue fluorescence of callose present in the pollen tubes. They were identified and counted at 200x magnification. Any pollen grains of different size or structure (wall sculpturing) were recorded as "foreign pollen" (APPENDIX 1).

Flowers were dissected and examined sequentially, in order of their arrangement on the inflorescence. Pistils were ranked according to age by numbering from the top of the spikelet downwards. Hence the youngest pistil bearing pollen equalled one and the value of the oldest pistil varied between 5 and 19, depending on the age and size of the inflorescence (FIG.1B).



**FIGURE 1B:** Inflorescence structure of Staberoha banksii. Flowers were ranked according to age, from the youngest pollen-bearing pistil (near the upper end of the spikelet) to the oldest pistil (at the base of the spikelet). Mature flowers are indicated by black dots, immature flowers by open circles. Fertile bracts are indicated by black lines and infertile bracts by a double line. The number of pollen-bearing pistils on a spikelet varied from 5 to 19, depending on the age (stage of maturation) and size of the inflorescence.

Germinating pollen grains could be identified by the tube emerging from the aperture and a "glassy" appearance caused by the differentiation of cells within the grain (FIG.2). Pollen tubes on young stigmas fluoresced brightly against the dark background, but those on older stigmas near the lower end of the spikelet were distinguished with less certainty.



FIGURE 2: Germinating pollen grains with pollen tubes attached (microgametophytes) on a stigma of S.banksii. Mag. X200.

Pollen tube counts give a conservative estimate of the microgametophyte population on each pistil for several reasons: Problems which were encountered while counting include the three-dimensional nature of the pistil which obscured tubes; fungal attack, which made it difficult to distinguish between hyphae and pollen tubes; and occasional weak fluorescence of the stigmatic tissue which created a streaky background to the fluorescent tubes. In addition I suspect that pollen tubes in the older pistils (i.e. those

near the base of the spikelet) were less distinct due to their age. They were rarely observed in older flowers, despite the fact that the ovule had enlarged considerably (indicating successful fertilisation). To be consistent I counted only those tubes which could be traced to a particular pollen grain. It must be stressed that the data obtained therefore do *not* represent the total microgametophyte population and should be considered as a very conservative estimate.

### Seedset

To check seedset the number of developing ovules were recorded for each spikelet. In the absence of direct cytological observation I could not be absolutely certain that the ovules had been fertilised. In most cases however, the ovules had enlarged considerably and the ovary wall had hardened and assumed a slight green colour. As they had undergone considerable development, I am fairly confident that these were immature fruits. Those which had not hardened or changed colour were dissected. If there was no visible endosperm formation or enlargement of the ovule, it was considered to be unfertilised. Seedset was expressed as a proportion (number of fertilised ovules/total number of developing ovules) as spikelets showed considerable variation in size (28-45mm) and maturity.

### Data analysis

A nested ANOVA for unequal sample sizes was used to determine whether the mean number of grains per pistil were significantly different between and within individual plants. The data were  $\log(x+1)$  transformed in order to meet the assumptions of an ANOVA (Sokal & Rolfe 1981).

To test whether pistils continue to entrain pollen throughout the flowering period, the number of grains were plotted against pistil number. It was predicted that the oldest pistils at the base of the spikelet would have a greater pollen load than those from flowers which had recently opened.

To investigate the effect of the total stigmatic area on the number of pollen grains entrained by a pistil, I compared the average pollen load on two-styled pistils to those with three styles. A Mann-Whitney test was used to determine whether these differences were significant.

To investigate the effect of distance from pollen source on the size of the pollen load occurring on pistils, the mean number of grains per plant was plotted against the distance to the nearest male. A simple linear regression was used on logged values to determine how much of the variation in pollen load size between plants was explained by distance to pollen source.

Staberoha banksii has a single ovule and therefore theoretically only needs a single microgametophyte to achieve fertilisation. To investigate the degree of potential competition between microgametophytes, the number of pistils with more than one germinating pollen grain (= microgametophyte) were expressed as a proportion of the total number of pollen-bearing pistils. Data were analysed only for the five youngest pollen-bearing stigmas as pollen tubes were difficult to count accurately on older pistils.

## RESULTS AND DISCUSSION

### SIZE OF THE POLLEN LOAD

The size of the pollen load varied widely among pistils, ranging from 0 to 196 pollen grains. Considerable variation was observed at three different levels - between plants, within plants (i.e. between spikelets on the same plant) and particularly within spikelets (i.e. between pistils on the same spikelet).

A nested analysis of variance showed that the mean number of pollen grains per pistil was significantly different both between plants and between spikelets on the same plant (TABLE 1). I therefore needed to examine within-plant variation before I could address differences between plants and relate these to distance from pollen source.

TABLE 1: Nested analysis of variance of the numbers of pollen grains occurring on pistils from 13 plants in a Staberoha banksii population.

SOURCE OF VARIATION	df	SS	MS	F	p
Among plants	12	62.5	5.21	20.49	<0.001
Within plants	51	13.0	.25	1.86	<0.001
Within spikelets	664	90.9	.14		

The amount of pollen occurring on a pistil is determined by two main factors: the availability of pollen (i.e. the amount of windborne pollen passing the stigma) and the efficacy of pollen capture by the stigma. Pollen availability is determined primarily by the distance from the pollen source and the size of the pollen source. The

relationship between distance from source and the concentration of airborne pollen however is dependent on atmospheric conditions such as windspeed and turbulence. Thus pollen density may be highly variable within a population.

The efficacy of pollen capture by a stigma may be influenced by the position of the spikelet on the plant (eg. its orientation with respect to the prevailing wind direction), the position of the flower on the spikelet, and the age of the stigma (i.e. the length of time it has been exposed to the airstream). TABLE 2 summarizes the factors influencing the size of the pollen load on a stigma. In the following discussion I will consider each factor *and see to what a to what* extent it accounts for the variation observed in numbers of pollen grains per stigma.

TABLE 2: Factors influencing the amount of pollen occurring on a stigma in Staberoha banksii.

<b>EFFICACY OF POLLEN CAPTURE</b>
1. Stigma age
2. Stigmatic surface area
3. Position of flower on spikelet
4. Position of spikelet on plant
<b>POLLEN AVAILABILITY</b>
5. Distance from nearest male plant
6. Size and density of the pollen source

## 1. Stigma age

Flowers in S. banksii mature acropetally. Stigmas at the base of the spikelet are therefore exposed to the airstream for a relatively longer period of time and can potentially accumulate more pollen than those which have recently opened. Stigmas were ranked according to their age from the youngest pollen-bearing pistil (no.1) to the oldest pistil situated at the base of the spikelet.

It was predicted that the oldest stigmas would have the greatest pollen loads, and the youngest the least, but only the latter trend was observed (FIG.3). The youngest pollen-bearing pistil on a spikelet typically had fewer than 5 pollen grains. The size of the pollen load tended to increase basipetally but varied enormously on older stigmas (i.e. pistil nos.6 to 18).

Although there is no consistent relationship between stigma age and pollen load size, the data indicate that stigmas do continue to entrain pollen throughout the flowering period. An important question related to stigma age is how long they are receptive. For example large pollen loads on old pistils which have accumulated over several days or weeks may be of little biological relevance if stigmas are receptive for only a short period of time. The significance of pollen load size in S. banksii can only really be interpreted once the duration of stigma receptivity is known. This could be obtained by determining how many stigmas are simultaneously receptive on the same spikelet (by using hydrogen peroxide and checking for bubble-formation). Observations made in this study indicate that the length of receptivity may be quite long, as newly germinated pollen grains (with pollen tubes only just emerging) were found on pistils of all ages (APPENDIX 1).

MEAN NO. OF POLLEN GRAINS

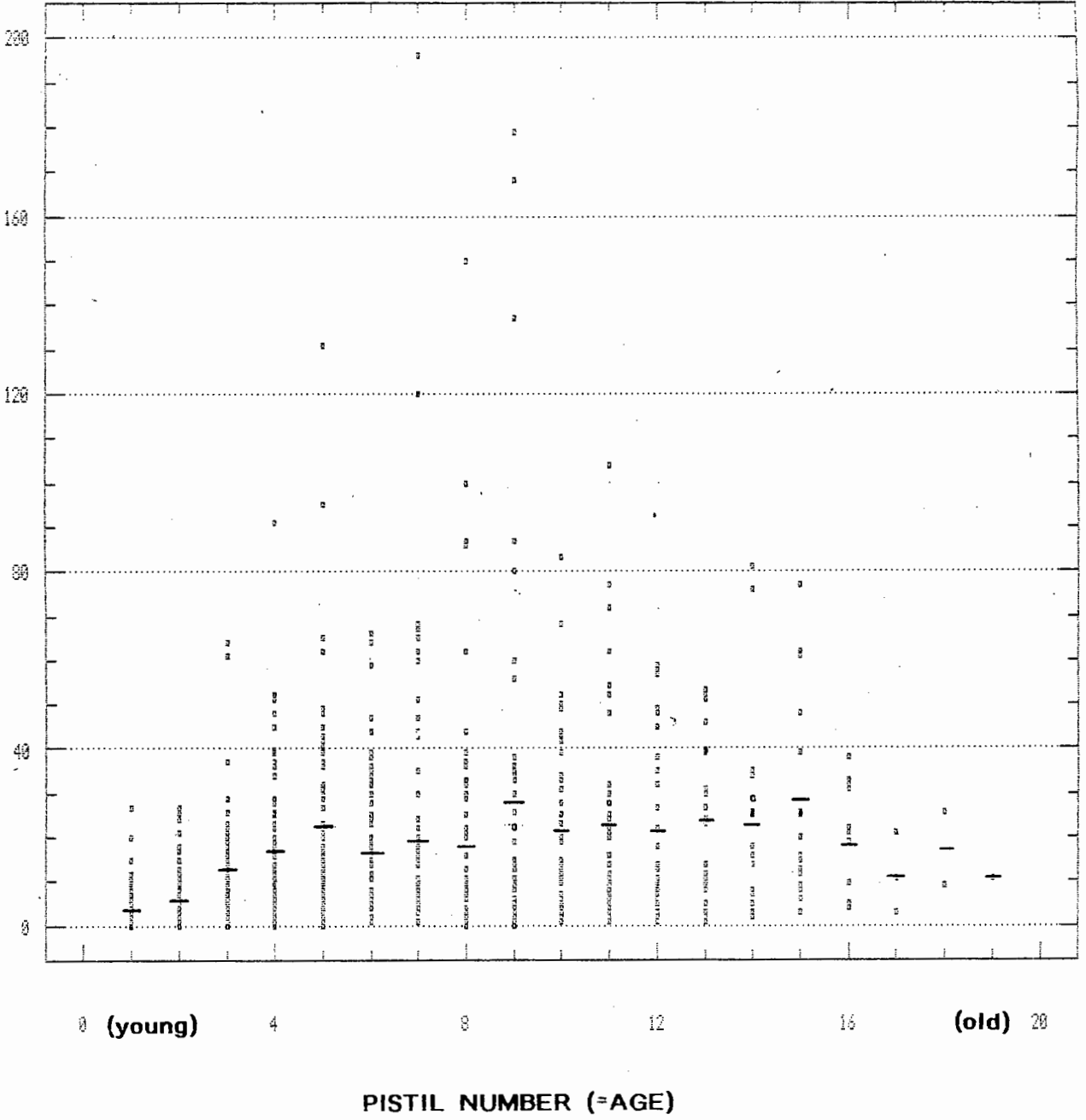


FIGURE 3: Distribution of pollen occurring on pistils of different ages from 64 spikelets of *Staberoha banksii*. Stigmas were numbered basipetally from the youngest pollen-bearing pistil (no.1) to successively older flowers on the floral axis. Horizontal bars represent the mean number of grains occurring on pistils of a given age.

## 2. Stigmatic surface area

Several authors have referred to the adaptive significance of a large, feathery stigma in wind pollinated plants (Whitehead 1969, Faegri & Van der Pijl 1979). ~~They suggest~~ They suggest that a larger surface area increases the efficiency of the stigma as it will filter a greater volume of air. The stigmatic surface area of S. banksii varies considerably among pistils as some have two styles and others are three-styled. To test whether the amount of pollen captured by a stigma is related to its surface area, I compared the mean number of pollen grains occurring on pistils with two and three styles. Results were unexpected as the mean number of pollen grains on pistils with two styles was 20.9, which was significantly greater than the mean pollen load of 12.1 grains found on pistils with three styles (Mann-Whitney large sample test statistic;  $Z = 5.51$ ;  $p < 0.001$ ,  $n = 706$ ).

There were significantly more two-styled pistils than those with three styles in a total sample of 706 flowers ( $p < 0.001$ , TABLE 3). One may infer from this that natural selection has selected for plants with two styles as these are most efficient at capturing pollen. The number of styles per pistil varied both between and within spikelets however, indicating that this is a quantitative trait and may not be genetically heritable. I would hesitate to invoke the role of selection until it has been shown that style number is an inherited trait, and that the aerodynamic behaviour of these two stigma morphologies is consistently different.

The stigma of S. banksii is atypical of wind pollinated plants because it is inserted and thus does not filter pollen directly from the air. It has been suggested that the subtending bract functions as a scoop to entrain pollen from the airstream (Linder, in press). The selective advantage of

a large stigmatic surface area may therefore be reduced in this species relative to plants which have exerted stigmas.

**TABLE 3:** Difference in the mean number of pollen grains occurring on pistils with two styles and three styles, and the relative frequency of each (pistils from 13 plants in a Staberoha banksii population at Cape Point).

	2-styled pistil	3-styled pistil
Total no. of pistils <sup>1</sup> (n = 706)	433	273
Mean no. of pollen grains per stigma <sup>2</sup>	20.9 ± 21.9	12.1 ± 18.8

1. Difference in the frequency of two and three-styled pistils sampled:  $\chi^2 = 36.47$ ,  $p < 0.001$ .
2. Difference in size of the mean pollen load: Mann-Whitney test,  $Z = 5.51$ ,  $p < 0.001$ .

### 3. Flower position on the spikelet

It was observed that the lowermost few flowers on the spikelet were frequently poorly developed and that these pistils often had fewer pollen grains than those situated higher up on the inflorescence. Both the maximum and the mean number of pollen grains per pistil show a tendency to tail off towards the base of the spikelet (FIG.3).

Interpretation of the distribution of pollen within a spikelet is marred by the fact that the number of pollen-bearing pistils varied from 5 to 19 among different

spikelets. Thus while pistil number 5 occurred at the base of one spikelet, a pistil of the same rank may have occurred on the upper end of another spikelet (FIG.1). Stigma age and position on the inflorescence therefore do not vary independently.

To test whether the position of the pistil had any effect on the amount of pollen captured, I compared the mean number of pollen grains on pistils from the upper, middle and lower region of the spikelet (TABLE 4).

**TABLE 4:** The mean number of pollen grains on pistils from different regions of the spikelet. The mean pollen load size of pistil numbers 1, 2 and 3 ("UPPER END") were compared to the lowermost two pistils at the base of the spikelet ("LOWER END"), and to the remaining pistils on the inflorescence ("MIDDLE REGION").

Position in spikelet	Mean + S.D.†	n
UPPER END	7.72 ± 9.38*	192
MIDDLE REGION	22.81 ± 25.55**	407
LOWER END	20.13 ± 21.49**	128

† 1-way ANOVA for significant differences between mean pollen load size ( $F = 32.29$ ,  $p < 0.01$ ). Stars indicate different subgroups (Tukeys range test).

Pistils at the upper end of the inflorescence (nos.1, 2 and 3) had on average half the number of pollen grains than those lower down on the spikelet. This result confirms the effect of pistil age on the size of the pollen load (discussed above). It was therefore expected that the lowermost two pistils at the base of the spikelet would have

the greatest pollen loads, as these have the longest period over which to accumulate pollen. They had fewer pollen grains on average than pistils in the middle region of the inflorescence, although the difference was not significant (TABLE 4).

There are two possible explanations why the lowermost two stigmas have smaller pollen loads: It may be due to the presence of two infertile bracts at the base of the spikelet which are rigid and overlap with the fertile bracts above. The latter subtend the flowers and open slightly when the flower is mature. The stiff infertile bracts may thus inhibit movement of the bracts situated just above and reduce the chance of pollen capture. Another possible reason is that pollen was lost from the older stigmas during the staining procedure. Pollen appeared to be "lodged" among the fine stigmatic branches however, and unattached pollen grains were very rarely observed.

#### 4. Spikelet position on the plant

A nested ANOVA indicated that within-plant differences in the size of the pollen load were statistically significant ( $p < 0.001$ , TABLE 1). Several factors related to the position of the spikelet on the plant may influence how much pollen is captured. These include the orientation of the spikelet with respect to the prevailing wind direction and its height above the ground. Inflorescences near the ground may receive less pollen due to filtration by the surrounding vegetation and boundary layer effects. Spikelets were collected randomly from different parts of the tussock, so the observed differences in pollen load cannot be related to specific positions on the plant. Nonetheless the variation observed between spikelets has important implications for sampling. A large number of inflorescences per plant should ideally be collected. Decisions on a suitable sample size

should take into account that the standard deviation was typically as large as the mean when five spikelets were sampled per plant (see FIG.4).

##### 5. Distance from pollen source

The average size of the pollen load differed significantly between plants ( $p < 0.001$ , TABLE 1). I wished to assess whether these differences were related to distance to the nearest pollen source, or simply due to random differences in pollen availability.

Much of the within-plant variation in pollen load size can be attributed to positional effects of the flower and the age of the stigma (discussed above). In order to relate differences between plants to pollen availability, the influence of these factors had to be eliminated or reduced. To remove the effect of pistil age I took the mean number of pollen grains on the five youngest pistils of each spikelet (i.e. near the upper end of the spikelet). This enabled me to look at the effect of distance to nearest male plant on the probability of pollen capture, and gave me greater confidence that differences between plants in the mean pollen load size were due to differences in the availability of pollen rather than factors influencing within-plant variation.

The mean pollen load on plants decreased rapidly with increasing distance to the nearest male plant and appears to show a leptokurtic distribution (FIG.4). However the regression of log mean number of grains per pistil against log distance to nearest male plant accounted for only 45% of the variation in pollen load between plants.

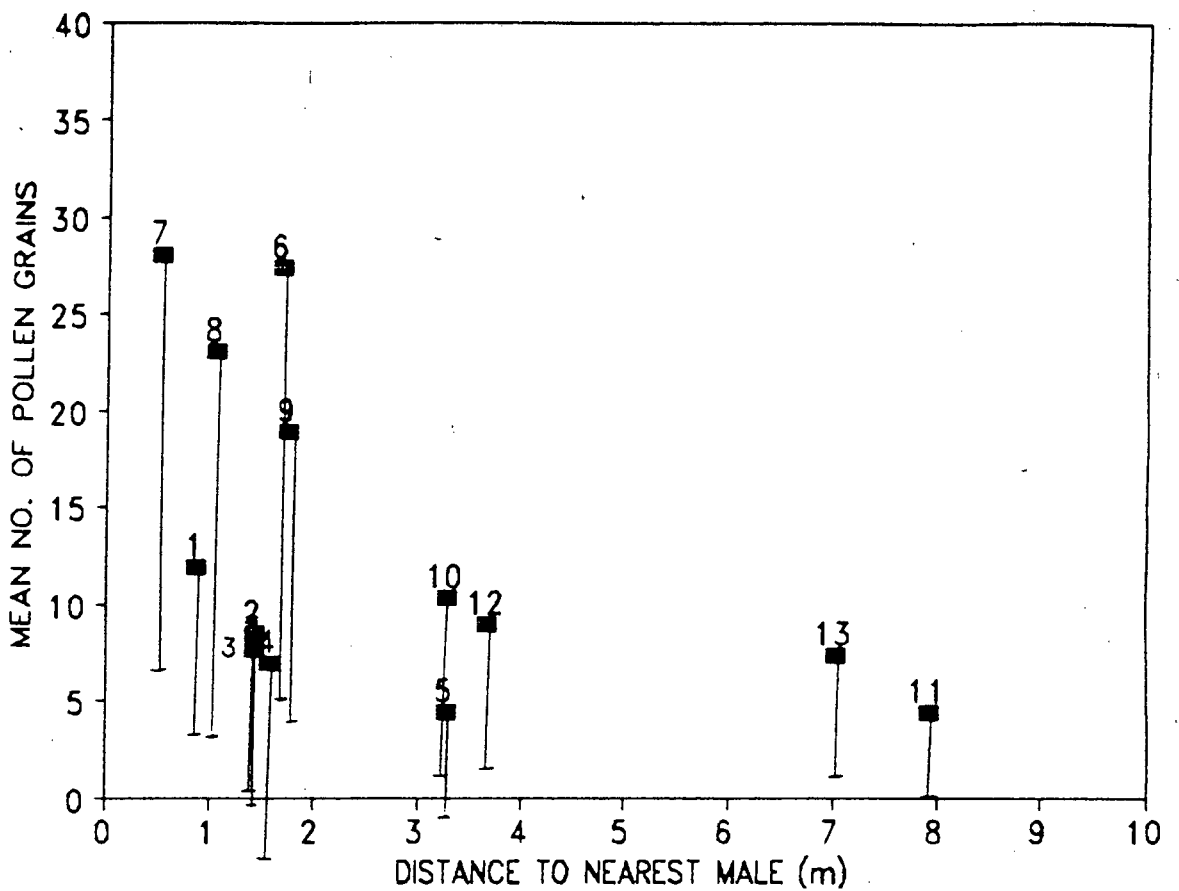


FIGURE 4a: Plot of mean pollen load size per plant (mean number of pollen grains per stigma) against distance to the nearest male plant (in meters). S.E. bars shown below the mean.

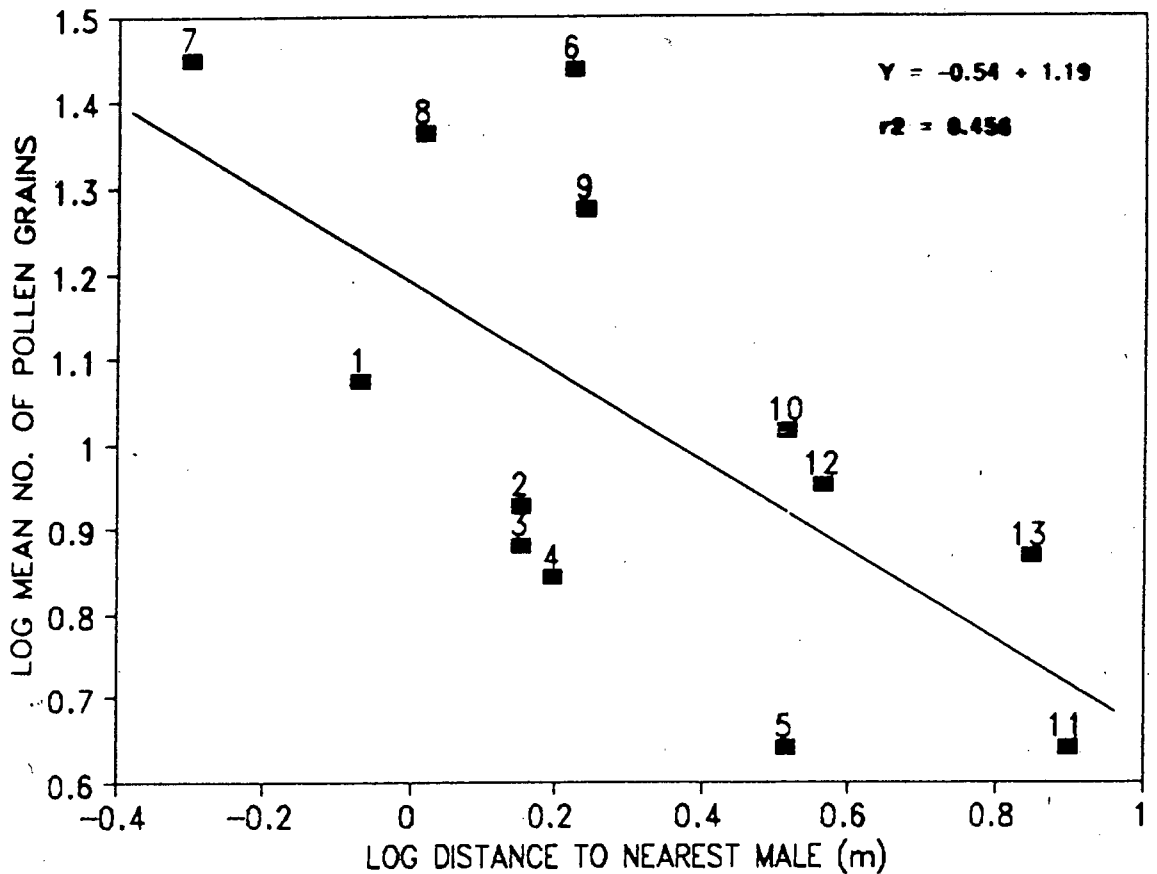


FIGURE 4b: A simple linear regression of log mean mean number of pollen grains per stigma per plant against log distance to nearest male plant (in meters).

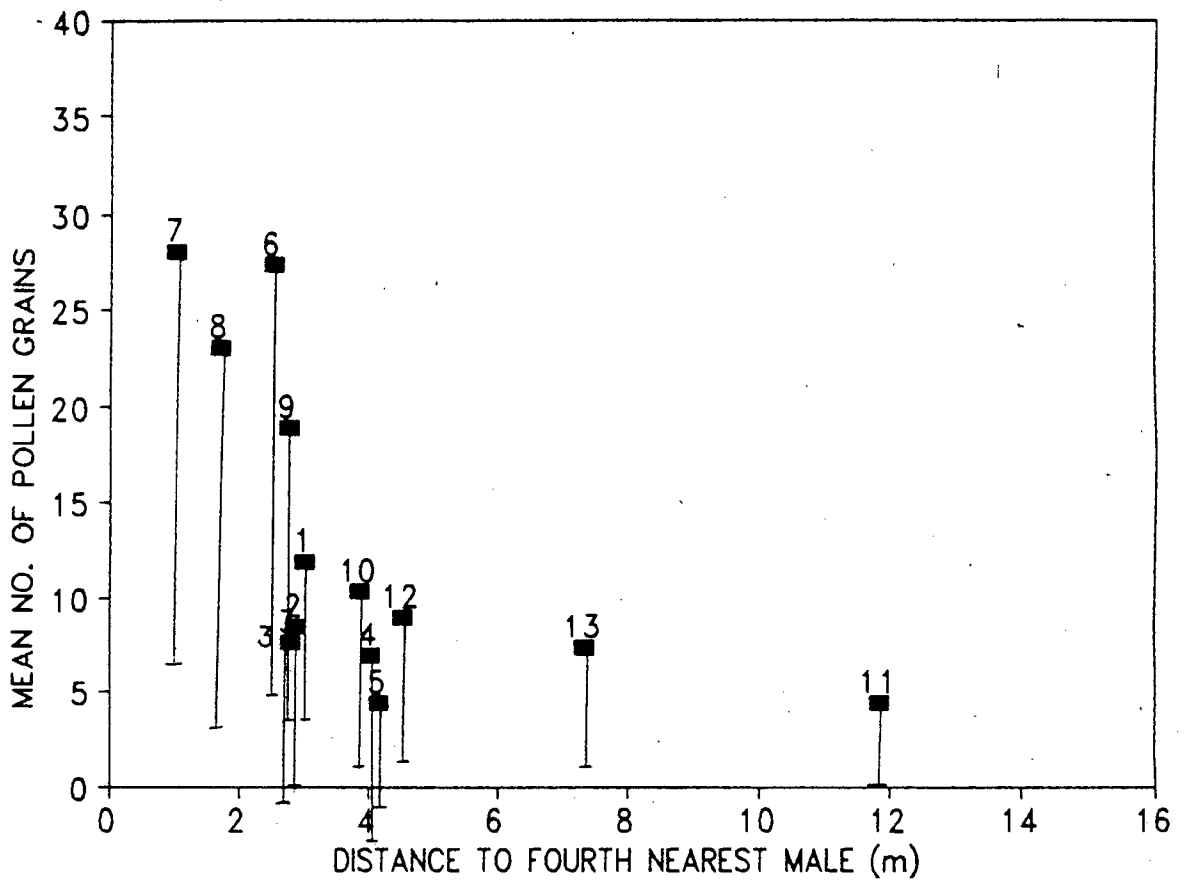


FIGURE 5a: Plot of mean pollen load size per plant (mean number of pollen grains per stigma) against distance to the fourth nearest male plant (in meters). S.E. bars shown below the mean.

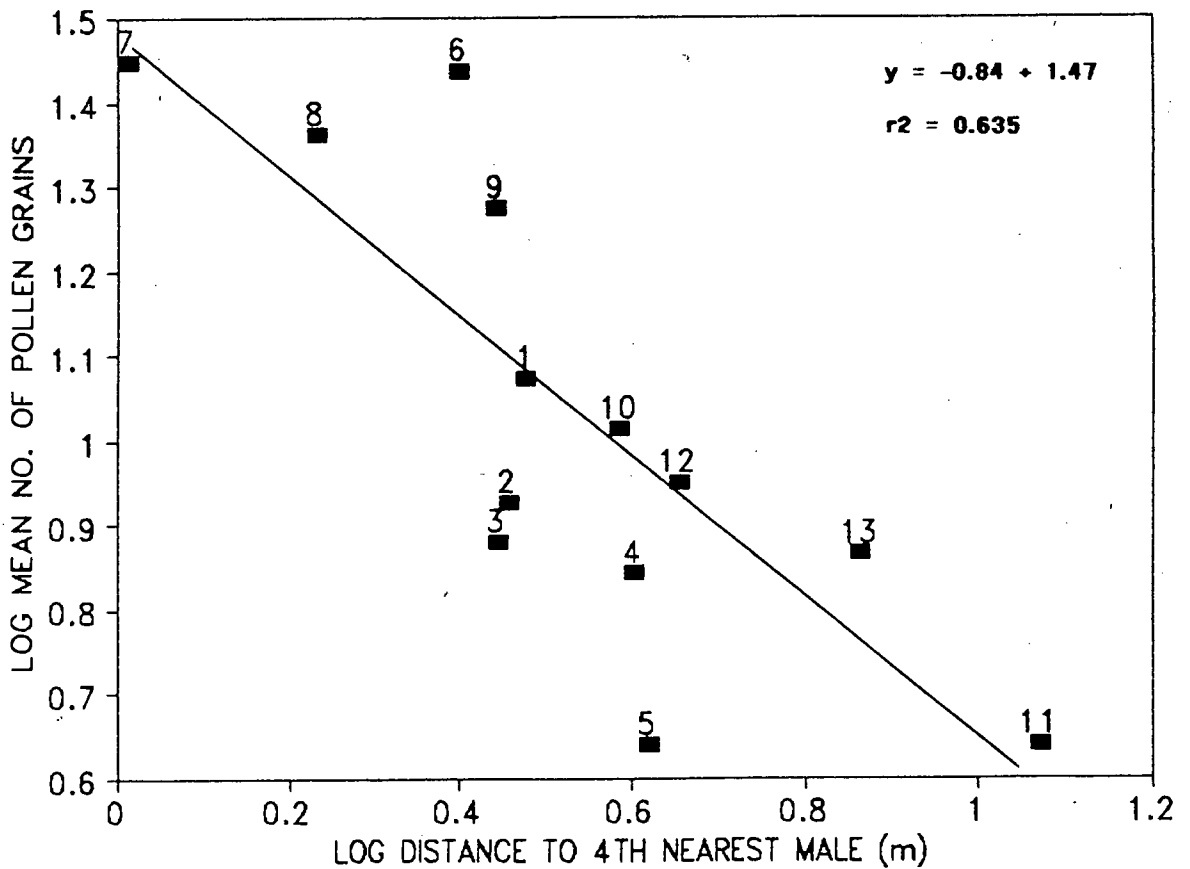


FIGURE 5b: A simple linear regression of log mean number of pollen grains per plant against log distance to the fourth nearest male plant (in meters).

There are problems with the use of nearest neighbour as a measure of pollen availability because it gives no indication of the size (density) of the pollen source. For example, no distinction is made between an isolated male tussock or a clump of male plants at the same distance. This is important as the dispersal characteristics for a single plant are quite different from those of a population (Levin & Kerstner, 1974). The problem of using nearest neighbour is that it implies a point source and emphasizes the leptokurtic character of pollen (Allison, 1990). For this reason plant density which emphasizes the diffuse origin of pollen may be a better measure.

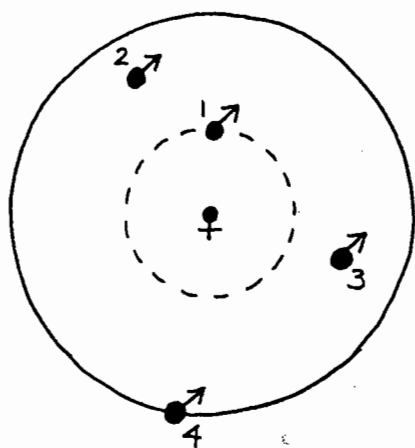
#### 6. Size and density of the pollen source

To investigate the effect of pollen availability on pollen load size, I required a measure of the local density of pollen surrounding a female plant. I therefore used the distance to the fourth nearest male plant, as this gave a better indication of the degree of isolation of a female plant than distance to nearest male. I regressed log mean pollen load size against log distance to fourth nearest neighbour. A simple linear regression explained 63.5% of the variation between plants in the mean number of pollen grains ( $p < 0.001$ , FIG.5). The value for  $r^2$  was considerably higher than that obtained for the regression using log nearest male plant ( $r^2_{4th\ nearest} = 0.635$  compared to  $r^2_{nearest} = 0.456$ ).

This result confirms that distance to the nearest neighbour may be an unsuitable measure to investigate the effect of distance from pollen source on the pollen load of a plant. Distance to the fourth nearest male included an indirect measure of abundance and therefore explained a greater proportion of the variance. FIG.6 illustrates how distance to the  $n^{th}$  nearest neighbour can overcome some of the problems associated with the use of distance to nearest

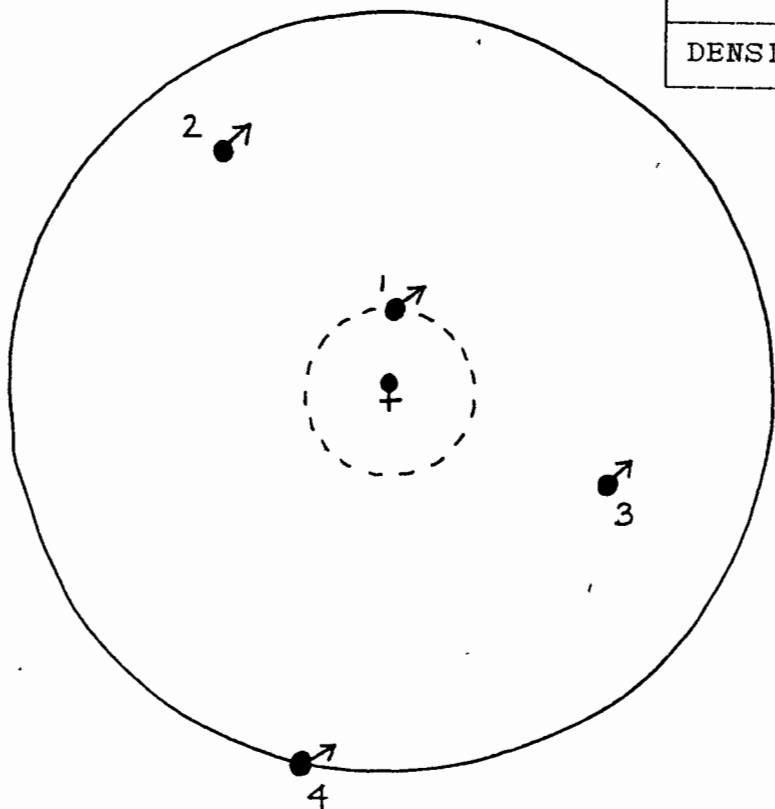
FIGURE 6: Diagram to illustrate the use of distance to nth nearest neighbour as a measure of the relative density of the pollen source. Measurement of distance to the nearest male plant only gives an indication of the proximity of the pollen source, and would not distinguish between the situations in A and B. Distance to the fourth nearest male gives an indirect measure of plant abundance and some indication of the density of the pollen source. (Plant density calculated for the area of the circle using the scale 1cm = 1m and multiplied by 10 000).

A



DISTANCE TO NEAREST MALE	1m
DISTANCE TO 4th NEAREST MALE	2.5m
DENSITY (plants/ha)	2037

B



DISTANCE TO NEAREST MALE	1m
DISTANCE TO 4th NEAREST MALE	5m
DENSITY (plants/ha)	509

neighbour as a measure of pollen availability. However, although it gives a reflection of male plant density in the area surrounding the female, this measure gives no indication of the proximity of the nearest pollen source. It may therefore be an inappropriate method to use over large distances or in populations where individuals are widely dispersed.

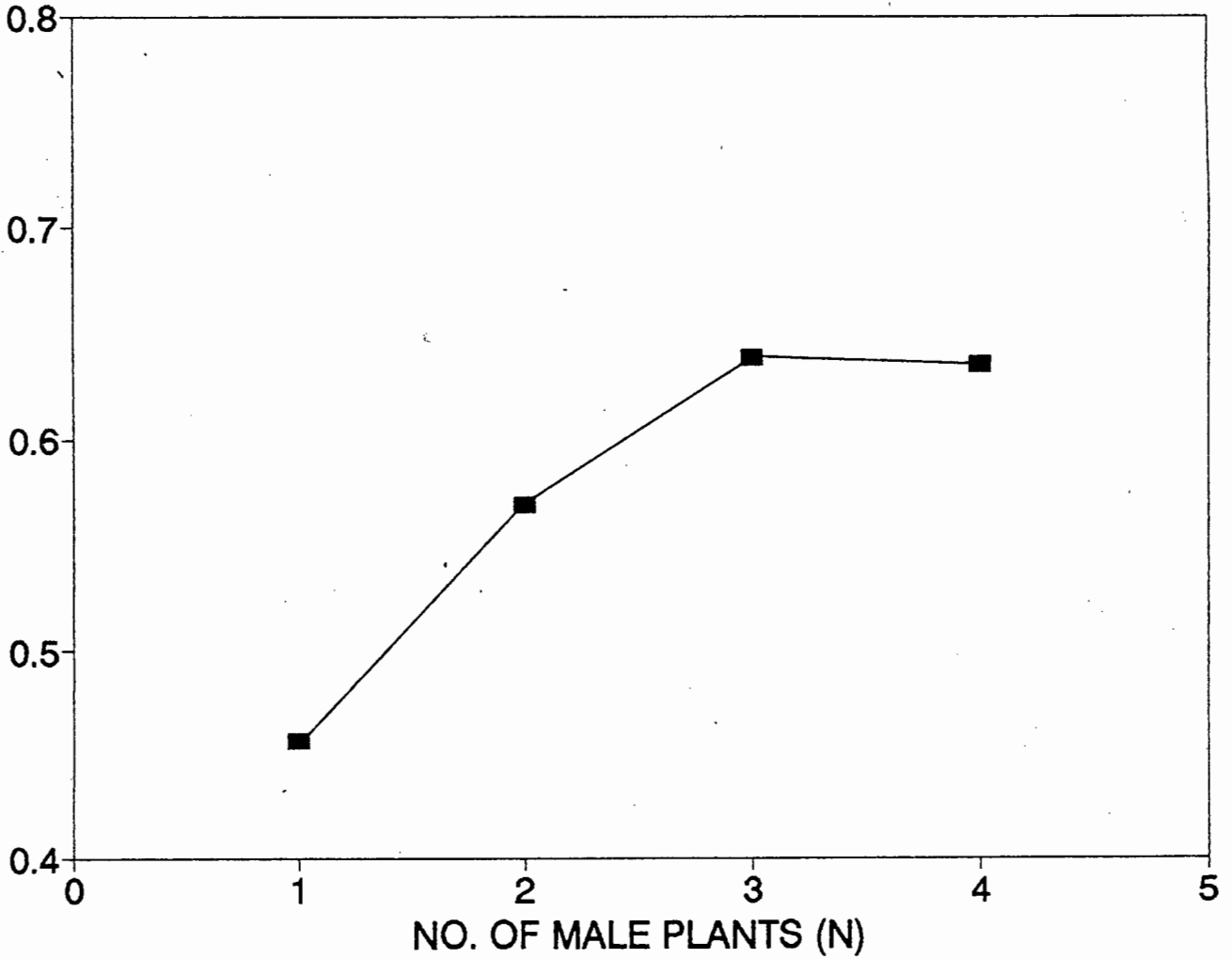
The use of distance to the fourth nearest male plant was an arbitrary choice determined by the sampling procedure. At what point does distance to successively further male plants cease to explain a greater proportion of the variance in pollen load size between plants? i.e. at what distance does an increase in the number of male plants fail to affect the amount of pollen captured by a female plant? How would one determine how many neighbours to include in future studies?

To address these questions I regressed log mean pollen load against log distance to nearest male, and the second, third and fourth nearest male plants respectively. The values for  $r^2$  of the four linear regressions give an indication of the amount of variation in mean number of pollen grains per plant explained by each male plant. I therefore plotted  $r^2$  against the number of male plants measured to see how it changed with each successively further neighbour (FIG. 7).

The value for  $r^2$  increases from 0.45 for the nearest male to 0.64 for the third nearest male and remains constant at 0.63 for the fourth nearest male plant. This indicates that the amount of pollen on these plants is a saturating function of pollen availability. Distance to the fourth nearest plant failed to explain any more of the variation in pollen load between plants than the third nearest male. Hence the distance to successively further neighbours explains an increasing proportion of the variation only up to a certain point. This "point" determines how many neighbours should be

FIGURE 7: The amount of variance in mean pollen load size per plant explained by distance to successively further male plants.  $r^2$  values were obtained from simple linear regressions of log mean number of pollen grains per plant against log distance to nearest male plant, and 2nd, 3rd and 4th nearest male plants respectively.

VARIANCE EXPLAINED BY DISTANCE TO Nth MALE ( $r^2$ )



measured and will depend on the density and dispersion (whether random or aggregated) of the population. The method described above for investigating the effect of distance is unusual as it considers the amount of pollen which was captured by stigmas rather than the distances over which pollen is dispersed. Although the results indicate that the probability of pollen capture is strongly limited by distance, it should be emphasized that these distances do not necessarily apply to pollen transfer.

#### NUMBERS OF MICROGAMETOPHYTES

The number of germinated pollen grains varied widely among stigmas, ranging from 0 to 45 for all the stigmas observed. 21.5% of the total amount of pollen counted on these plants had germinated and 66% of the stigmas had at least one microgametophyte. These values considerably underestimate the situation in reality due to methodological problems (see Methods) and because pollen grains on the youngest pistil of each spikelet had not had a chance to germinate before sampling. The following discussion on competition between microgametophytes therefore considers only the five youngest pistils on each spikelet.

Staberoha has a single ovule, hence competition could theoretically occur in those pistils with more than one microgametophyte. 66% of the pistils had two or more microgametophytes and pistils with more than 20 pollen tubes growing down the style were not uncommon (TABLE 5). On average there were 5.19 microgametophytes per ovule (geometric mean = 7.99). There is therefore considerable potential for gametophytic competition. Pollen-pollen competition is thought to lead to gametophytic selection and result in greater progeny fitness (Snow 1986). Although the ecological and evolutionary significance of this process has

been explored in theory, there is little evidence at present that pollen genotype is expressed in the phenotype (Snow & Mazer, 1988). The degree of gametophytic competition could also be considered simply as a measure of the probability of successful pollination. Pistils with a large number of pollen tubes growing down the style are more likely to be fertilized than those with fewer microgametophytes.

Few studies have determined the size of microgametophyte populations in *natural* populations and these have all been done on insect or hummingbird pollinated species (Snow 1986, Levin 1990). The available evidence indicates that gametophytic competition in these plants is not intense: On average there were only 2 microgametophytes per ovule in Epilobium canum (Snow 1986), 4.73 microgametophytes per ovule in Phlox drummondii and roughly 3 per ovule in Polemonium viscosum (Levin 1990 and references therein).

This is the first study to my knowledge which has determined the size of microgametophyte populations in a wind pollinated species. The mean number of microgametophytes is greater than the biotically pollinated species referred to above, largely because Staberoha has only one ovule per pistil (i.e. not necessarily because the pollen loads were much larger). The large amounts of ungerminated pollen is perhaps unexpected in an obligately outcrossing plant. Some of the pollen grains were shrunken and appeared inviable, but the majority were hydrated and undamaged. Failure to germinate could be due to lack of stigma receptivity, pollen viability or possibly incompatibility.

TABLE 5: Numbers of microgametophytes occurring on pistils of Staberoha banksii at Cape Point. The mean and range are given for pollen tubes counted in pistils from 13 plants (n = 25)

Plant no.	Pollen tubes(N) Mean $\pm$ SD	Range	% pistils with N = 0	% pistils with N > 2
1*	1.76 $\pm$ 2.35	0 - 9	52	40
2	7.20 $\pm$ 6.68	0 - 27	8	76
3	3.20 $\pm$ 4.20	0 - 15	44	52
4*	2.36 $\pm$ 4.37	0 - 19	56	32
5	1.52 $\pm$ 1.63	0 - 6	28	36
6	8.85 $\pm$ 7.88	0 - 27	5	64
7	6.48 $\pm$ 6.02	0 - 20	8	76
8	6.56 $\pm$ 5.69	0 - 23	16	80
9	7.96 $\pm$ 5.59	0 - 21	4	96
10	8.16 $\pm$ 6.82	0 - 22	8	80
11	3.36 $\pm$ 2.99	0 - 11	20	68
12	5.84 $\pm$ 5.41	0 - 19	12	68
13	4.96 $\pm$ 3.87	0 - 16	4	84

\* Pollen tubes in these stigmas flouresced very faintly and could not be distinguished from surrounding stigmatic tissue.

### SEEDSET

As inflorescences were sampled only 11 weeks after the peak flowering period, the ovules near the top of the spikelet were not fully developed. In the absence of cytological evidence I could not be sure if this was because the ovules were immature, or because they were unfertilised. Seedset was therefore determined only for the flowers occurring below the uppermost mature seed on the spikelet.

Seedset was high, the number of mature seeds ranging from 5 to 18 per spikelet. There was no significant difference in seedset in plants which were near to a pollen source and plants which were "far" (95,3% compared to 91,9%).

Individual plants situated 7 to 13 metres away from the nearest male plant showed no decline in seedset relative to those which had four male plants within a metre. This indicates that pollen dispersal by wind is highly effective within the population. The quantity of pollen captured by plants therefore does not limit fertilisation to any marked degree. This result influences our interpretation of the leptokurtic decline in pollen load size observed in plants sampled in May. The smaller pollen loads found on female plants which were isolated from male plants by up to 8 meters therefore do not necessarily mean the probability of fertilization is decreased. The relationships between distance, pollen transfer and pollen-pistil compatibility are potentially complex and require further investigation.

## CONCLUSIONS

Two aspects of this study limit the conclusions which can be drawn: First, only thirteen plants were sampled, of which few were isolated from male plants by more than 4 metres. Thus although these results may indicate a trend regarding the size of pollen loads per plant and the availability of pollen, further work is required to quantify the relationship. A second consideration is that the patterns of variation described may be peculiar to the population which was studied. Cape Point is an exceptionally windy area and it is possible that pollen dispersal distances are considerably higher than in other areas which experience less wind. Although pollen transfer is increased in high winds, we do not know how the efficacy of pollen capture is affected by windspeed.

A problem encountered throughout this study was that of scale. Variation was observed at three levels: within the population, within plants and within the spikelet. It was therefore difficult to interpret the observed patterns and to detect the underlying mechanism for these different levels of variation. Within-plant differences in pollen load size were attributed to factors influencing the efficacy of pollen capture. Although stigma age, positional effects of the spikelet and stigmatic surface area were considered, it is not known to what extent these contribute to the observed variation.

Between-plant variation is due to differences in pollen availability, but the relative importance of the density and proximity of neighbouring male plants is not clear. Pollen load size showed a leptokurtic distribution indicating that the probability of *pollen capture* is reduced with increasing distance to the pollen source. The distances mentioned in this study however do not apply to the dispersal of pollen

which may be considerably greater. An important difference between this study and other studies done on wind pollination is that I quantified the amount of pollen captured by Staberoha plants rather than the amount of pollen which is dispersed. Results therefore required a different method of interpretation and allowed me to investigate the efficiency of wind pollination from a new angle. Further studies are needed to see if the observed patterns in the numbers of pollen grains and microgametophytes are representative of other populations and if they apply to other members of the Restionaceae.

The large numbers of pollen grains and microgametophytes and the high seedset observed in S.banksii indicate that the quantity of pollen is not limiting fertilization in this population, and that wind is a very effective pollen vector. This suggests we may severely underestimate the efficacy of wind pollination and need to re-examine the common conception that anemophily is a wasteful, inefficient process.

Although I attempted to address specific questions concerning the pollination of S.banksii more questions and untested ideas were generated than results. It is hoped that these will stimulate further work on wind pollination in the Restionaceae and that this study may contribute towards establishing an improved methodology.

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## APPENDIX 1:

The numbers of pollen grains, microgametophytes and foreign pollen occurring on stigmas of Staberoha banksii. Spikelets were sampled from 13 plants occurring in a population at Cape Point (May 1990).

SUMMARY STATISTICS FOR THE COMPLETE DATA SET: (MEAN  $\pm$  S.D.)

No. of pistils:	727	
No. of pollen grains:	13 366	18.39 $\pm$ 22.61
No. of microgametophytes:	2875	3.95 $\pm$ 5.30
No. of foreign pollen:	236	1.96 $\pm$ 1.76

21.51% of the total number of pollen grains had germinated. 1.73% of the pollen was "foreign" (i.e. not belonging to S. banksii).

(PLEASE TURN OVER)

Plant	Spikelet	Pistil#	Styles	Grains	Tubes	Foreign
1	1	1	2	1	0	
1	1	2	2	3	1	
1	1	3	2	15	0	2
1	1	4	2	19	3	
1	1	5	2	23	0	1
1	1	6	2	16	2	
1	1	7	2	22	4	3
1	1	8	2	36	4	2
1	1	9	3	8	0	2
1	1	10	2	33	3	10
1	1	11	2	6	0	1
1	1	12	2	18	0	3
1	1	13	3	46	0	5
1	1	14	3	7	0	
1	1	15	3	16	0	5
1	1	16	3	5	0	3
1	1	17	2	11	0	
1	2	1	2	3	1	
1	2	2	3	8	3	
1	2	3	2	14	4	
1	2	4	2	8	0	
1	2	5	2	32	0	
1	2	6	2	6	0	
1	2	7	1	14	0	
1	2	8	3	37	0	
1	2	9	3	23	0	
1	2	10	3	25	0	
1	2	11	3	20	0	
1	2	12	2	22	0	
1	2	13	3	31	0	
1	2	14	3	16	0	
1	2	15	3	15	0	
1	2	16	3	5	0	
1	3	1	2	6	0	
1	3	2	3	18	9	
1	3	3	3	21	2	
1	3	4	2	13	0	
1	3	5	2	16	0	
1	3	6	3	33	0	
1	3	7	3	11	0	
1	3	8	3	21	0	
1	3	9	3	8	0	
1	3	10	2	23	0	
1	3	11	3	28	0	
1	3	12	2	4	0	
1	3	13	3	11	0	
1	3	14	2	5	0	
1	3	15	3	9	0	
1	3	16	3	22	0	
1	3	17	2	3	0	
1	4	1	2	9	5	
1	4	2	2	7	4	
1	4	3	2	17	0	3
1	4	4	3	22	0	
1	4	5	3	20	0	1

1	4	6	2	30	0	
1	4	7	2	47	12	
1	4	8	2	30	0	
1	4	9	2	26	0	
1	4	10	2	20	0	
1	4	11	2	24	0	
1	4	12	2	45	0	
1	4	13	3	24	0	4
1	4	14	2	26	0	2
1	4	15	3	20	0	
1	4	16	2	33	0	2
1	4	17	3	21	0	1
1	4	18	3	9	0	2
1	4	19	2	11	0	1
1	5	1	2	3	2	
1	5	2	2	0	0	
1	5	3	2	5	5	
1	5	4	2	13	5	
1	5	5	2	1	0	
1	5	6	2	17	6	
1	5	7	2	7	2	1
1	5	8	2	9	0	
1	5	9	2	13	4	
1	5	10	2	23	0	
1	5	11	2	3	1	
1	5	12	2	27	2	
1	5	13	2	3	0	
1	5	14	2	14	0	
1	5	15	2	39	1	
1	5	16	2	10	0	
<hr/>						
2	1	1	2	5	5	
2	1	2	3	6	5	
2	1	3	2	9	7	
2	1	4	3	19	16	
2	1	5	2	14	11	1
2	1	6	2	4	2	
2	2	1	2	1	1	
2	2	2	2	2	2	
2	2	3	2	15	12	
2	2	4	2	20	18	1
2	2	5	2	18	15	
2	3	1	2	1	1	
2	3	2	2	7	6	
2	3	3	2	13	9	
2	3	4	2	18	15	
2	3	5	3	29	27	
2	3	6	3	30	26	
2	3	7	2	10	8	
2	4	1	2	1	1	
2	4	2	2	4	3	
2	4	3	3	4	4	
2	4	4	2	4	4	
2	4	5	2	0	0	
2	5	1	2	1	1	
2	5	2	2	0	0	
2	5	3	2	3	2	

2	5	4	2	10	9	
2	5	5	2	7	6	
2	5	6	3	12	11	1
3	1	1	2	5	4	
3	1	2	2	11	9	
3	1	3	2	3	0	
3	1	4	2	8	0	
3	1	5	2	0	0	
3	1	6	3	12	0	
3	1	7	2	15	0	
3	1	8	2	16	12	
3	1	9	2	3	3	
3	1	10	2	10	0	
3	1	11	2	1	1	
3	1	12	2	7	0	1
3	1	13	3	30	2	1
3	2	1	2	6	6	
3	2	2	2	0	0	
3	2	3	2	0	0	
3	2	4	2	13	0	
3	2	5	2	9	0	
3	2	6	2	16	0	
3	2	7	2	16	0	2
3	2	8	2	3	0	
3	3	1	2	3	3	
3	3	2	2	1	0	
3	3	3	2	2	2	
3	3	4	2	14	12	
3	3	5	2	3	2	
3	3	6	2	20	13	
3	3	7	2	6	3	
3	3	8	2	7	5	
3	3	9	2	14	6	
3	4	1	2	0	0	4
3	4	2	1	3	3	1
3	4	3	2	21	15	19
3	4	4	2	34	7	3
3	4	5	2	10	4	1
3	4	6	2	7	0	1
3	4	7	2	7	3	
3	5	1	2	11	10	
3	5	2	2	17	0	2
3	5	3	2	6	2	2
3	5	4	2	9	0	
3	5	5	2	1	1	
3	5	6	2	24	15	
3	5	7	2	5	4	
3	5	8	2	0	0	
3	5	9	2	0	0	1
3	5	10	1	0	0	
4	1	1	2	1	0	
4	1	2	3	0	0	
4	1	3	2	0	0	
4	1	4	3	3	0	
4	1	5	2	4	0	
4	1	6	2	1	0	1

4	1	7	2	4	0	
4	1	8	3	2	0	
4	1	9	2	1	0	
4	1	10	3	1	0	
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4	2	3	1	1	0	
4	2	4	2	3	0	
4	2	5	2	1	0	
4	2	6	2	8	0	
4	2	7	3	3	0	
4	2	8	2	2	0	
4	2	9	2	5	0	
4	2	10	3	10	0	
4	2	11	2	4	0	
4	2	12	2	3	0	
4	3	1	2	4	3	
4	3	2	3	17	5	
4	3	3	2	16	19	
4	3	4	3	23	0	
4	3	5	2	12	0	
4	3	6	2	23	0	3
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4	3	8	2	8	0	
4	3	9	2	5	0	
4	3	10	3	6	0	
4	3	11	2	5	0	
4	3	12	2	5	0	2
4	3	13	2	1	0	
4	4	1	2	1	1	
4	4	2	2	1	1	
4	4	3	2	2	0	
4	4	4	2	0	0	
4	4	5	2	9	0	
4	4	6	2	1	0	
4	4	7	2	7	0	
4	4	8	3	4	0	
4	4	9	2	11	0	
4	4	10	3	3	0	
4	5	1	2	4	3	
4	5	2	2	7	5	
4	5	3	3	2	1	
4	5	4	2	17	9	
4	5	5	2	41	10	
4	5	6	1	15	0	
4	5	7	3	8	0	
4	5	8	3	11	3	
4	5	9	2	11	6	
4	5	10	2	7	0	
5	1	1	3	3	3	
5	1	2	2	0	0	
5	1	3	2	1	1	2
5	1	4	3	0	0	1
5	1	5	3	1	1	
5	1	6	3	2	2	
5	1	7	3	4	4	2

5	5	15	3	3	0	
5	5	16	3	5	0	
6	1	1		20	18	
6	1	2		21	8	
6	1	3		64	1	
6	1	4		11	0	
6	1	5		131	7	
6	1	6		12	1	
6	1	7		19	1	
6	1	8		150	3	
6	1	9		137	13	
6	1	10		52	0	
6	1	11		21	1	
6	2	1		1	1	
6	2	2		6	5	
6	2	3		13	12	
6	2	4		39	4	
6	2	5		17	6	
6	2	6		35	6	
6	2	7		15	3	
6	2	8		36	3	
6	2	9		80	1	
6	2	10		34	3	
6	2	11		62	0	
6	3	1	2	1	1	
6	3	2	2	6	6	
6	3	3	2	11	7	
6	3	4	1	39	26	
6	3	5	2	65	27	
6	3	6	2	47	23	2
6	3	7	3	196	45	
6	3	8	3	86	3	
6	3	9	3	168	7	
6	3	10	3	19	2	
6	3	11	3	104	3	
6	3	12	2	14	2	
6	4	1	3	3	2	
6	4	2	2	7	6	
6	4	3	3	9	6	
6	4	4	3	36	16	1
6	4	5	2	39	18	1
6	4	6	2	39	15	
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