Bot. Bull. Academia Sinica (1992) **33:** 33-42

Flowering of *Ziziphus mucronata* subsp. *mucronata* (Rhamnaceae): anthesis, pollination and protein synthesis

P. C. Zietsman[1,3] and F. C. Botha[2]

[1]*National Museum, P.O. Box 266, Bloemfontein, Republic of South Africa*
[2]*Department of Botany, University of the Orange Free State, Bloemfontein, 9300, Republic of South Africa*

(*Received December 26, 1990; Accepted August 16, 1991*)

Abstract. The self incompatible, monomorphic flowers of *Ziziphus mucronata* are protandrous and dichogamous. Dichogamy is complete and the flower can furthermore be regarded as heterodichogamous. The use of hydrogen peroxide, guaiacol and peroxidase activity to indicate stigma receptivity, as was assessed morphologically during anthesis, proved inadequate. The use of protein synthesis rate as determined with pulse labelling of stigmatic tissue, is proposed as a sensitive, relatively easy and reliable method for indicating stigma receptivity. Six different gene products were detected unique to receptive stigmatic tissue. The polypeptides which are induced in receptive stigmatic tissue are not glycoproteins with D-mannose or D-glucose as carbohydrate residues. Four glycopolypeptides with molecular weights of 63.4, 57.7, 54.4 and 40 kilodalton, however, are present at all stages in the stigmatic tissue.

Key words: Anthesis; Breeding system; Floral physiology; Protein synthesis; Rhamnaceae; *Ziziphus*.

Introduction

Dichogamy, a breeding system which encourages outcrossing (Baker, 1983), is divided into five different classes on the bases of pollen and stigma presentation; the floral units involved; the degree of separation in presentation of pollen and stigma; the average interval between these two functions and the degree of synchrony of blossoms within one plant (Webb and Lloyd, 1986). The excistence of two genetical morphs which differs in timing of anthesis during the day has furthermore been described (Galil and Zeroni, 1967).

Mechanisms that prevent inbreeding and ensures outcrossing genetically in self-incompatible species are the sporophytic and gametophytic incompatibility systems. In the former S-proteins have been related to the appearance of glycoproteins on the stigma which coincide with the development of self-incompatibility (Roberts *et al.*, 1979; Ferrari *et al.*, 1981; Hinata *et al.*, 1982; Takayama *et al.*, 1986). These glycoproteins have molecular weights of approximately 51-57 kD and are S-proteins, bounded with sugars such as D-mannose and D-glucose. In the gametophytic self-incompatibility system, where pollen tube growth is inhibited in the style, S-proteins with molecular weights of approximately 32 kD have been described (Anderson *et al.*, 1986).

Substances present in the stigma and/or style which can be used in determining receptivity and which are also apparently related to the process of incompatibility, include esterase (Mattsson *et al.*, 1974; Heslop-Harrison and Shivanna, 1977; Shivanna *et al.*, 1978; Vithanage, 1984; Gaude and Dumas, 1986; Murray, 1986), peroxidase (Bredemeijer, 1974; 1979), glycoproteins, amino-acids and glycosphingolipids (see Van Went and Willemse, 1984 for review). Gaude and Dumas (1986) have demonstrated, cytochemically, ATPase and adenylate cyclase activity in the pellicle of *Brassica* stigmas. However, there does not exist a

[3]To whom correspondence should be addressed.

sensitive and reliable quantitative method for assessing stigma receptivity. The aim of this study was therefore to develop such a method as well as to determine the breeding system of *Ziziphus mucronata* Willd. subsp. *mucronata* (subsequently referred to as *Z. mucronata*).

Materials and Methods

Material

Flowers, floral parts and pollen of *Z. mucronata* were collected at the study area at Glen Agricultural College, South Africa (28 26 Cd). All chemicals used in preparation for and during electrophoresis were obtained from Merck Chemicals. The chemicals used in the fluorochromatic work were obtained from Saarchem, South Africa. Radio - active amino acids (^{3}H-leucine and ^{35}S-methionine), cocktail, Amplify and X-ray film were obtained from Amersham. All other chemicals used were of the highest purity available. Nitrocellulose (0.45 μm pore size) was obtained from Amersham and gold labelled Concanavalin-A (20 nm) from the Sigma Chemical Company. Tritium labelled leucine (1.7 to 2.6 TBq • mmol^{-1}) and ^{35}S-methionine (> 37 TBq • mmol^{-1}) were obtained from Amersham.

Anthesis

The nature of anthesis in *Z. mucronata* was determined by monitoring 40 different individuals at the study area; from 05h00 to 20h00. Movement of the sepals, petals and stamens as well as pistil development were closely monitored at 15 minute intervals.

Controlled Pollination Experiments, Pollen Germination and Pollen Tube Growth

Controlled pollination experiments were conducted as described by Gunatilleke and Gunatilleke (1984) and Harvey and Braggins (1985). Pollen tube growth and germination were determined *in vivo* by a technique based upon the fluorescence of callose (Martin, 1959; Jefferies and Brain, 1984). Pistils fixed in FAA were rinsed in tap water and transferred to a 3.5% (w/v) commercially available sodium hypochlorite solution (Gillet's Javel), to soften and clear the tissues. The softened pistils were soaked in tap water for at least one hour to remove most of the bleach. Thereafter the material was stained in a 0.1% (w/v) solution of water-soluble aniline blue in 0.33 M K_3PO_4 for at least 10 min. The stained pistils were then mounted and squashed in staining medium on glass slides and viewed under ultraviolet light. A Nikon Labophot with a fluorescent facility and appropriate filters was used.

Qualitative Measurement of Peroxidase/Catalase Activity

To determine the onset of stigma receptivity, a drop of a 5% (v/v) H_2O_2 solution was carefully placed on an open flower, so as to cover the stigma. Flowers in the six different stages of anthesis were used. The release rate of oxygen bubbles, a result of the reduction of H_2O_2 to water and oxygen by peroxidase on the stigma were subjectively evaluated by awarding a 1 (low rate of oxygen release), a 2 (medium rate of release) or a 3 (high rate of oxygen release).

Alternatively, flowers in the different stages of anthesis were immersed in 10% guaiacol. The colour change on the stigmas was subjectively evaluated after 10 minutes.

Total Peroxidase Activity

A modified method of Johnson-Flanagan and Owens (1985) was used for the extraction and measurement of total peroxidase activity. The peroxidase was extracted in a 50 mM potassium phosphate buffer (pH 6.0). The stigmas and styles of 10 flowers of each of the different stages of anthesis, were homogenized with a polytron (87/Polytron PTA 20S) for 90 seconds at 4°C in 600 μl of the buffer. Triton X-100 was then added to the extract to a final concentration of 0.15% (v/v) and then thoroughly mixed. The extract was left, on ice, for 5 minutes and then centrifuged at 10,000 g for 5 min at 4°C. The standard assay for peroxidase contained in a final volume of 1 ml, 40 mM phosphate buffer (pH 6.0), 88 mM H_2O_2 and 0.46 mM guaiacol. The assay was started by addition of the H_2O_2. Changes in absorbance at 470 nm were recorded using a Phillips UV/VIS Scanning spectrophotometer (PU 8720).

Extraction of Total Proteins

Extraction of total proteins from *Z. mucronata* flowers for SDS-PAGE proved to be a major problem. Both extraction methods involving buffered aqueous solutions, as well as extraction with organic solvent prior to aqueous extraction, were used. Of these, extracts with an aqueous Tris-buffer were found to be optimal and were therefore used during extraction of total proteins. In all cases the stigma and style of 10

flowers were excised and then homogenized in 600 μl extraction medium.

The extraction buffer contained 12.5 mM Tris-HCl (pH 6.8) containing 10% (v/v) glycerol and 5% (w/v) 2-mercaptoethanol. Extracted proteins were precipitated by adjusting the extract to 15% (w/v) TCA and allowing at least 60 minutes at 0°C for quantitative precipitation. After centrifugation at 9,500 g for 5 minutes the resulting pellet was washed three times in 500 μl 100% (v/v) acetone (-20°C). The residual acetone was removed from the pellet under partial vacuum.

In all cases precipitated proteins were dissolved in a sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 5% (w/v) 2-mercaptoethanol and 2.3% (w/v) SDS. The protein extract was heated for 5 minutes at 90°C to facilitate protein denaturation before electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Polypeptides were resolved on 12% polyacrylamide gels with a 6% stacking gel (ratio of acrylamide to N'N'-methylene-bis-acrylamide 37.5:1). The running gel contained 0.36 M Tris-HCl (pH 8.8), 0.1% (w/v) SDS, 0.04% (w/v) ammonium persulfate and 0.04% (v/v) TEMED. The 6% stacking gel contained 12.4 mM Tris-HCl (pH 6.8), 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and 0.078% (v/v) TEMED. Before polymerization was induced by adding TEMED, the solutions were thoroughly degassed under partial vacuum. The running buffer consisted of 25 mM Tris-HCl (pH 8.3), 19.2 mM glycine and 0.1% SDS. Separated polypeptides were electrophoretically transferred from unstained gels to nitrocellulose membranes at 100 V for 1 h in a Biorad Transblot cell containing 25 mM Tris, 192 mM glycine and 20% (v/v) methanol.

Staining of Total Polypeptides

Colloidal gold with a mean particle diameter of 15 nm was prepared by reducing chloroauric acid (gold chloride) with citrate. This was done by boiling 0.01% (w/v) chloroauric acid in 0.04% (w/v) trisodium citrate for approximately 45 minutes. Total polypeptides on the nitrocellulose filters were stained as follows: The nitrocellulose filters were rinsed for 15 minutes in 50 mM Tris-HCl (pH 6.8) containing 0.1% (v/v) Tween-20. The colloidal gold solution (pH 6.0 to 6.5) was then poured onto the membrane and then incubated with continuous agitation for 15 to 90 minutes. The reaction was stopped by rinsing the filters thoroughly in the Tris-Tween buffer. Silver enhancement of the gold stain was obtained by washing the nitrocellulose filters after the gold binding with a solution containing 1.28% (w/v) citric acid monohydrate and 1.17% (w/v) trisodium citrate for at least 30 minutes. Hereafter the nitrocellulose filters were soaked for 10 minutes without shaking in 25 ml of a solution containing 4.5% (w/v) trisodium citrate, 5.1% (w/v) citric acid monohydrate, 0.022% (w/v) silver lactate and 0.17% (w/v) hydroquinone. The filters were then thoroughly rinsed with distilled water and fixed with commercially available photographic fixative.

Staining of Glycoproteins

The method of Dubray and Bezard (1982) was used for glycoprotein staining. After protein blotting, the nitrocellulose filters were quenched in 50 mM Tris-HCl (pH 7.8) containing 0.05% (v/v) Tween-20 and 2% (w/v) BSA for 30 minutes with agitation. The filters were then incubated overnight in a 1:100 dilution of gold labelled Con-A in 0.05% Tween (v/v), 0.15 M NaCl, 0.01 M sodium phosphate, 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$ (pH 7.0). Unbounded Con-A was removed by washing the filter five times with PBS-Tween for 30 minutes. Glycoproteins were then visualized by silver enhancement.

In vivo Protein Synthesis

Five fresh stigmas and styles of each of five stages of anthesis (B_1, B_3, C_1, C_2, C_3) were collected and incubated in 5 μl aqueous solution stabilized with 0.05 (v/v) mercaptoethanol, 25 mM pyridine, 3,4-dicarboxylic acid, 444 kBq ^{35}S-methionine and 370 kBq ^{3}H-leucine. This volume was adequate to completely submerge the stigmas. After incubating the material for 2 h at ambient temperature the tissue was rapidly frozen by submerging it in liquid nitrogen.

Extraction of Labelled Proteins

Total proteins were extracted as already described. To ensure complete precipitation of the proteins 10 μg of unlabelled BSA was added to each extract before precipitation with TCA. The precipitated proteins were washed twice in -20°C acetone and dried under partial vacuum. The pelleted proteins were dissolved in the sample buffer. Radio-activity was determined with a LKB Wallace 1217 Rack Beta scin-

tillation counter. Insta-Gell II was used as a scintillation cocktail. Counts were corrected for quenching, using the external standard method.

Fluorography

Gels containing separated polypeptides were treated and infiltrated with Amplify according to the instruction of the manufacturer. After drying with a Biorad, Model 443 Slab Dryer at 60°C for one hour, the gels were exposed to X-ray film (Hyperfilm-MP).

Densitography

The presence and relative abundance of polypeptides were quantified by using a Hoefer GS 300 Transmittance/Reflectance Scanning Densitometer. Gels and autoradiographs were scanned in the absorbance mode while nitrocellulose blots were scanned in the reflectance mode. Data was analyzed with the Hoefer GS 365 Data System software package.

Results

Anthesis

Individual trees of *Z. mucronata* can be divided into early, and late flowering groups (Fig. 1). Anthesis starts at 07:00 a.m. in the early flowering group (group A), and at 11:00 a.m. in the late flowering group (group B). In approximately 85% of the trees in the population anthesis starts in the early morning. The nature of anthesis is the same in these two groups. Anthesis can be divided into three main phases (Fig. 1). The first is an asexual phase, lasting approximately 2 h, followed by a male phase which lasts for up to 4 h and a female phase which may last for 21 h. On grounds of mor-

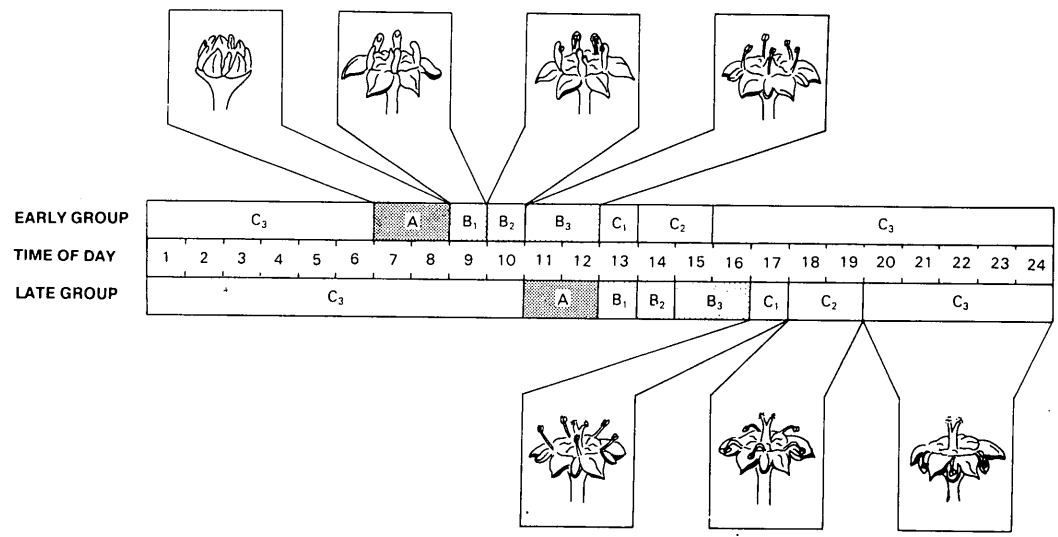

Fig. 1. Schematic representation of different timing of anthesis in the two groups of *Ziziphus mucronata* flowers over a period of 24 h.

ASEXUAL PHASE

A: Flowers start to open.

MALE PHASE

B_1: Flowers partly opened. Stamens perpendicular to flower base, but still covered by petals. Pollen inaccessible to insects. Style short and stigmas undeveloped.

B_2: Flowers fully opened. Petals starting to diverge. Pollen accessible to pollinators. Styles start to elongate. Drops of nectar visible on disc.

B_3: Petals fully diverged. Stamens devoid of pollen. Disc covered by thin layer of nectar. Stigmas start to develop.

FEMALE PHASE

C_1: Stamens start to diverge. Stigmas almost fully developed. Droplets visible on stigmas.

C_2: Stamens almost fully diverged.

C_3: Stamens fully recurved. Pistil fully developed.

phological changes, anthesis can be subdivided into seven shorter stages (Fig. 1). The longevity of an individual flower is approximately 26 h.

Controlled Pollination Experiments, in vivo Examination of Pollen Germination and Pollen Tube Growth

None of the flowers that were artificially self pollinated set seed. Pistils of these flowers that were examined under UV light revealed that a large number of pollen grains germinated on the stigmatic surface. Pollen tube growth, however, was arrested during growth through the style (P.C. Zietsman, unpublished).

Catalase and Peroxidase Activity

The release of oxygen bubbles was observed during all stages of anthesis from those parts of the flower which came into contact with the H_2O_2. Oxygen release was most intense from damaged areas, such as the margins of the petals. Althoug difficult to quantify, it was possible to use a stereo microscope to observe the rate of oxygen release from the stigmatic surface. Increase of oxygen release was detected between the male stages B_1 and B_2 and followed by a further increase between the male stage B_3 and the female stage C_1. The rate of oxygen release remained constand during the three female stages C_1 to C_3.

Stigmatic surfaces of flowers in the male phase of anthesis turned brownish after being immersed in guaiacol for 10 minutes, while those flowers in the female phase turned into a much darker brown colour.

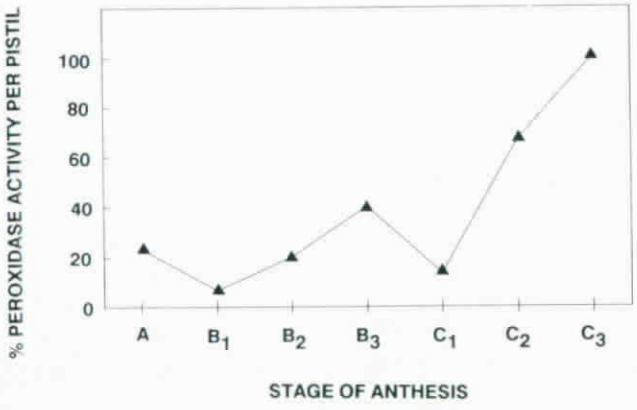

Fig. 2. Peroxidase activity, as a percentage of activity during stage C_3, of stigmatic and stylar tissue of *Ziziphus mucronata* flowers during the different stages of anthesis.

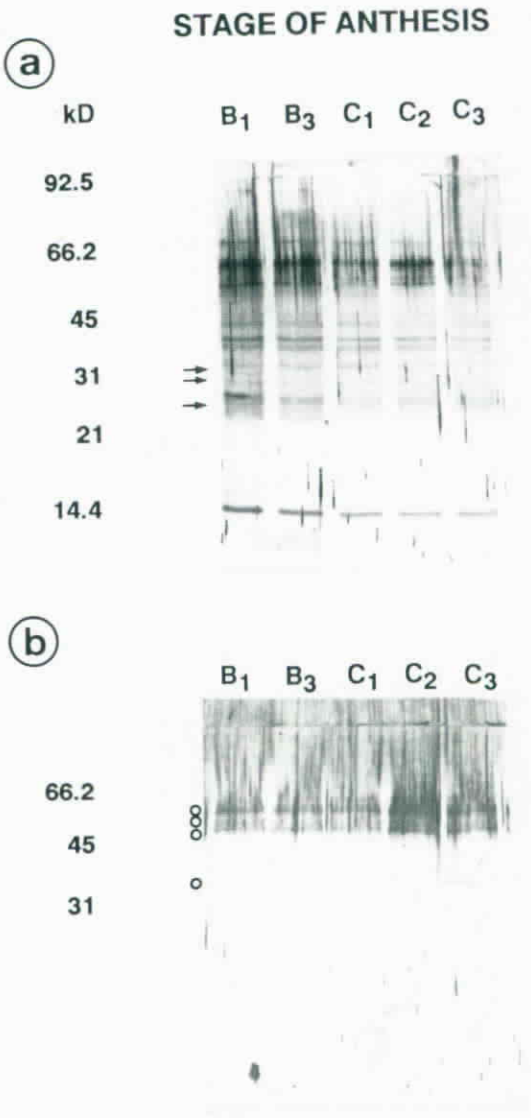

Fig. 3. Electrophoretic analysis of stigmatic and stylar polypeptides of *Ziziphus mucronata* flowers in the different stages of anthesis, on SDS–PAGE (12% running gel and 6% stacking gel with a 37.5:1 acrylamide to N'N'-methylene-bis-acrylamide ratio). Total polypeptides were transferred to nitrocellulose before staining (see text for details). a: silver enhanced gold staining for total proteins; b: gold labelled Concanavalin A staining for glycoproteins. Molecular weight markers used were phosphorylase B (92.5 kD), BSA (66.2 kD), ovalbumin (45 kD), carbonic anhydrase (31 kD), trypsin inhibitor (21 kD) and lysozyme (14.4 kD). → = proteins apparently associated with male stages; ○ = glycoproteins present during all stages.

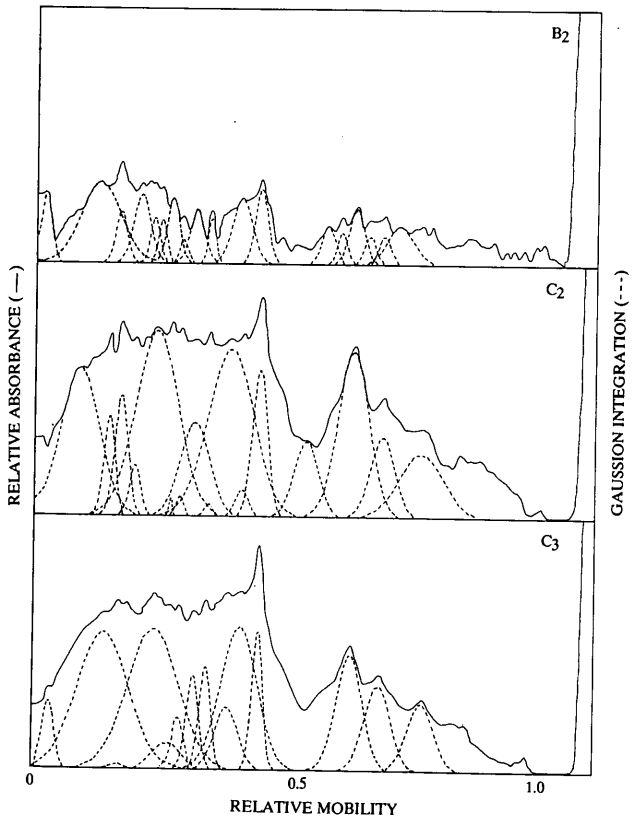

Fig. 4. Densitograph of stigmatic and stylar polypeptides of *Ziziphus mucronata* flowers in the different stages of anthesis, on SDS-PAGE after periodic acid-silver staining for glycoproteins (see text).

Damaged areas, for instance petal margins and anthers, as well as stigmas of flowers well past the C_3 stage also turned brown.

Due to the problems associated with quantifying as well as localization of peroxidase activity total peroxidase was measured spectrofotometrically. Peroxidase activity in the tissues of the stigma and style (Fig. 2) increased during the male phase of anthesis and peaks at stage B_3. Surprisingly, the activity decreased during transition form stage B_3 to C_1, then increased dramatically thereafter.

Total Cellular Polypeptides

Due to a limited source of available material staining with Coomassie R250 was unsuccessful and only a few polypeptides could be detected. Although silver staining works better than Coomassie staining, the results were still unsatisfactory (results not shown). The highly sensitive gold staining technique with which as little as 10 pg of a polypeptide can be detected after silver enhancement (Fig. 3a) was used.

It is evident that major differences in the total polypeptide pattern could be detected between the different stages of anthesis. For example, certain polypeptides present in stage B_1 are either absent from the female stages, or present in very low concentrations e. g. three polypeptides of approximately 35 kD, 30 kD and 29 kD (Fig. 3a). In order to determine if any glycoproteins are related to receptivity, gels were stained with a periodic acid silver stain. Major differences in polypeptide concentration is evident between the different stages of anthesis. This is indicated by the much higher concentration of the polypeptides during the C_2 stage as compared to the B_2 and C_3 stages (Fig. 4). However, none of the polypeptides were found to be unique to C_2. Total polypeptides transferred to nitrocellulose filters were also probed with gold labelled Con-A. Four polypeptides reacted with the Con-A and these appear to be present in all stages of anthesis (Fig. 3b). The molecular weights of these glycopolypeptides are 63.4 kD, 57.7 kD, 54.4 kD and 40 kD, respectively.

Protein Synthesis and Receptive Proteins

Protein synthesis during the male stages of anthesis was apparently very low (Fig. 5). The rate, however, increased during the female stages and reached its highest peak during the C_2 stage, whereafter it decreased again during the C_3 stage. Six labelled polype-

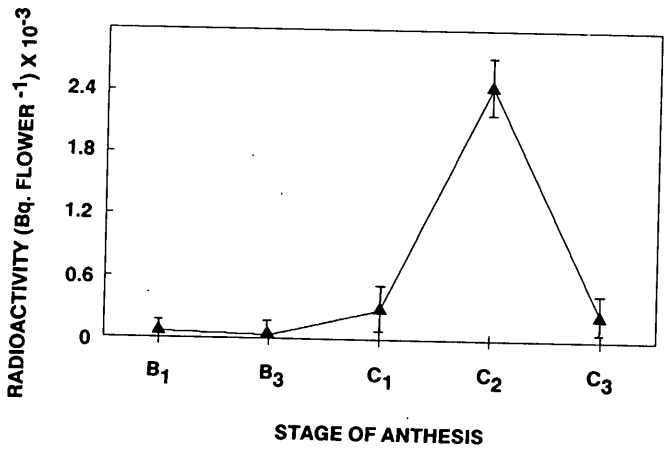

Fig. 5. Rate of protein synthesis of stigmatic and stylar tissue of *Ziziphus mucronata* flowers during the different stages of anthesis.

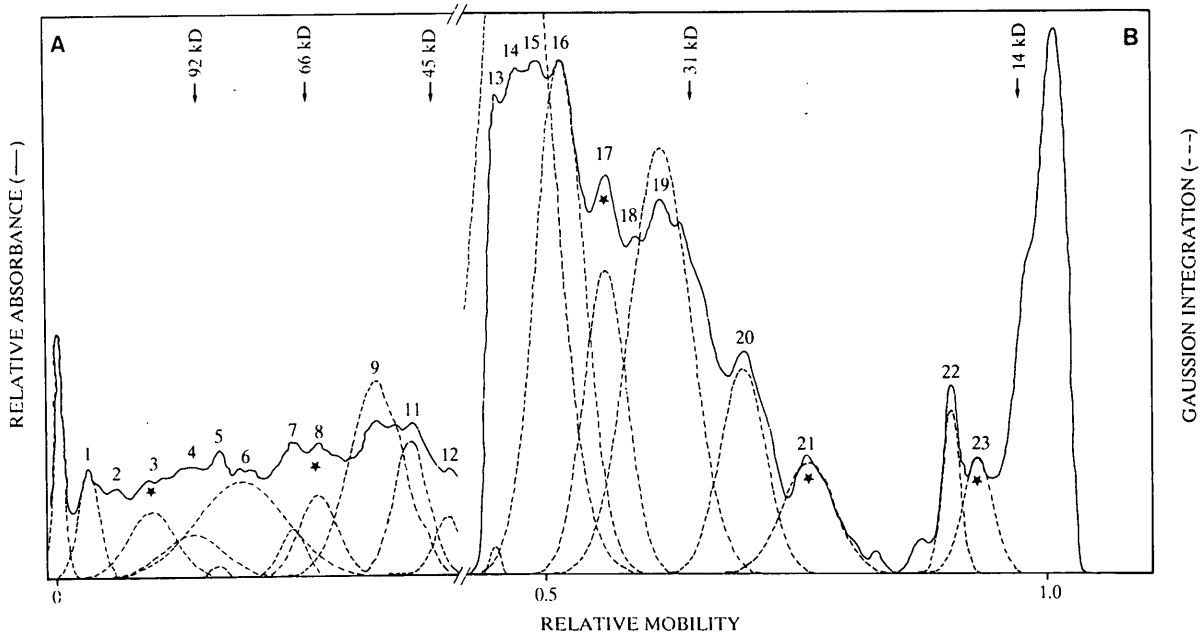

Fig. 6. Densitograph of the ^{35}S-methionine and ^{3}H-leucine labelled polypeptides of the stigmatic and stylar tissue of *Ziziphus mucronata* during different stages of anthesis. Polypeptides were analised on a 12% SDS-PAGE. Autoradiogram was scanned and peak areas integrated (see text). Due to large differences in labelling between high and low molecular weight polypeptides, an individual sample could not be scanned at the same sensitivity throughout. A = high molecular weight polypeptides (>46 kD) scanned at low sensitivity; B = low molecular weight polypeptides (<46 kD) scanned at high sensitivity. (peak 10 absent-only present in stage C_3).

ptides can be identified unique to C_2 (Table 1 & Fig. 6). The molecular weights of these polypeptides, were 16 kD, 23 kD, 24 kD, 37 kD, 68 kD and 104 kD respectively. A single polypeptide, unique to C_3, is also visible (Table 1).

Discussion

The evidence obtained in this investigation on the anthesis of *Z. mucronata*, shows the reproductive organs of this species are temporally rather than spatially separated. The monomorphic flowers of this species are thus dichogamous. Furthermore, the appearance of anthers before stigmas indicate protandry. The temporal separation of pollen and receptive stigma in *Z. mucronata* is presented in a single flower and can be regarded as intrafloral dichogamy. The lack of overlap between pollen presentation and stigma receptivity indicates dichogamy to be complete (Lloyd and Webb, 1986). The existence of what seems to be two genetical morphs in the population which differ in the timing of anthesis, as is the case in *Ziziphus spina-christi* (L.) Willd. (Galil and Zeroni, 1967), would according to Lloyd and Webb (1986), suggests that this species is heterodichogamous.

Laboratory conducted tests, in conjunction with hand pollination in the veld, indicates that *Z. mucronata* is self-incompatible and does not reproduce by apomixis. It is evident that the incompatible pollen hydrated and germinated normally. Pollen tube growth, however, was arrested in the stylar tissue indicating that this species has a gametophytic self-incompatibility system (Anderson *et al.*, 1989). Normally such a system is controlled by the action of a single multiallelic gene, but more complex systems involving two or more loci are known (Anderson *et al.*, 1989; Larsen, 1977; Lundqvist, 1964; Osterbye, 1975).

According to Faegri and Van der Pijl (1979) the release of oxygen from hydrogen peroxide by catalase has been used as a receptivity criterion. It was found in this study that oxygen release increased during anthesis and apparently reached a maximum at stage C_1.

Table 1. *Percentage areas of peaks integrated from a densitograph of the ^{35}S-methionine and ^{3}H-leucine labelled polypeptides of the stigmatic and stylar tissue of* ***Ziziphus mucronata*** *during different stages of anthesis*

Peak no	Stages during anthesis		
	B_1	C_2	C_3
1	5.7	4.0	3.7
2	8.8	a	3.8
3		5.4[b]	
4	3.0	4.4	e
5	e	0.3	9.4
6	9.1	14.8	1.6
7	7.4	2.0	11.5
8		5.4[b]	
9	a	18.6	16.5
10			4.1[d]
11	a	8.4	4.4
12	16.5	2.8	a
13	2.4	0.1	e
14	7.6	13.2	a
15	3.2	a	16.0
16	2.3	6.0	a
17	e	3.1[b]	0.6
18	6.3	a	e
19	3.7	6.2	11.7
20	e	2.2	2.5
21		1.4[bc]	
22	a	0.7	1.1
23		0.8[b]	

a=Polypeptide present but not integrated due to bad peak fit.
b=Polypeptides unique to C_2.
c=Two polypeptides present but integrated as a single peak.
d=Polypeptides unique to C_3.
e=Polypeptides absent.

Several problems in using this technique were experienced. Firstly, due to the small flowers, it was difficult to locate the exact spot of oxygen release. Secondly, it appeared that any damage to any part of the flower resulted in an increased oxygen release and thirdly, quantification of the released oxygen was impossible. Using the dye guaiacol, it was evident that peroxidase activity was not restricted to only the stigma. Colouration of both pistils and stigmas were evident. However, the amount of stain deposited on the stigma increased during stages B_1 to C_2 and C_3. As with the oxygen release determination, the lack of quantification made it impossible to determine whether differences in peroxidase reaction rates were present between stages C_2 and C_3.

The results obtained by determining peroxidase activity reflect total activity and therefore could be misleading. It should be kept in mind that peroxidase activity most probably reflects cellular damage during anthesis such as during opening of the stigma. Hence the relatively high peroxidase activity at stages A, B_3 and C_2/C_3. Furthermore, as it is known that increased peroxidase activity is a feature of cellular senescence (Grover and Sinha, 1985), it is not surprising that peroxidase activity increases dramatically during the late female stages of anthesis.

The fact that all the molecular weight standards were stained with the periodic acid silver stain clearly shows that the stain is non-specific. Due to this non-specificity it is not possible to conclude that the stained polypeptides are glycoproteins. Probing protein blots with Con-A did not reveal any clear differences between the different stages. However, previous studies have indicated that the S-proteins are usually induced in the stigmatic tissue 24 h or more prior to anthesis (Nasrallah, 1989). The molecular weights of two of the glycoproteins in the stigmatic tissue of *Z. mucronata* are similar to the S-related glycoproteins from other species (Roberts *et al.*, 1979; Ferrari *et al.*, 1981; Hinata *et al.*, 1982; Takayama *et al.*, 1986). It is also evident that these glycoproteins are abundant in the stigmatic tissue during all the described flowering stages.

From the data obtained when flowers were pulse labelled at different stages of incubation, it is evident that the rate of protein labelling during stage C_2 (receptive stage) is much higher than at any other stage of anthesis. The morphological identified receptive stage therefore precisely correlates with the maximum protein synthesis. Although this could indicate enhanced translation of existing mRNA, a much more plausible explanation is that total levels of mRNA increases substantially at stage C_2, due to increased gene expression. However, the possibility that changes in the rate of proteolysis could also contribute to the observed phenomenon cannot be ruled out. Due to the sensitivity and relative ease of this method, its possible use as an indicator of receptivity in other systems is most probably one of the major contributions of this study to future research in receptivity studies.

Fractionation of the labelled polypeptides clearly showed that the higher rate of methionine and leucine incorporation is not only due to an increase in total protein synthesis. Six different polypeptides, which are unique to C_2, can be distinguished. It is evident, however, that these polypeptides are not glycoproteins with D-mannose or D-glucose as terminal carbohydrate residues. Furthermore, these polypeptides were not stained with the periodic acid-silver stain. Although the function of these induced polypeptides is unknown, it is conceivable that they could merely be linked to changes in metabolism occurring at stage C_2.

The results obtained with the polypeptide profiles and protein synthesis clearly show that major changes occur in gene expression in the *Z. mucronata* stylar tissue during anthesis. The most interesting observation is that certain genes are obviously preferentially expressed during the male stages, while others are only expressed at the female stages associated with maximum stigma receptivity. Those gene products which are only present during the male stages are most probably linked to the rapid growth during elongation of the stylar tissue.

Although many aspects investigated in this study should merely be regarded as an introductory investigation, several very important conclusions can be made. It is evident that the morphologically identified stage of stigma receptivity also represents the stage of maximum protein labelling in the stigma. The potential use of protein synthesis as a criteria for stigma receptivity in tissues where morphological identification of receptivity is not possible could be a valuable tool.

Acknowledgements. We are indebted to the Directors and Council of the National Museum, Bloemfontein, R.S.A. and the Foundation for Research Development for financing this research as well as the Director of Glen Agricultural College for allowing us to do the fieldwork at Glen.

Literature Cited

Anderson, M. A., E. C. Cornish, S. L. Mau, E. G. Williams, R. Hoggart, A. Atkinson, I. Bonig, B. Grego, R. Simpson, P. J. Roche, J. D. Halley, J. D. Penschow, H. D. Niall, G. W. Tregear, J. P. Coghlan, R. J. Crawford, and A. E. Clark. 1986. Cloning of Cdna for a stylar glycoprotein associated with self-incompatibility in *Nicotiana alata*. Nature **321**: 38-44.

Anderson, M. A., P. R. Ebert, M. Altschuler, and A. E. Clarke. 1989. Molecular genetics of self-incompatibility in flowering plants. *In* E. Lord and G. Bernier (eds.), From floral induction to pollination. American Society of Plant Physiologists, Maryland, USA, pp. 165-173.

Baker, H. G. 1983. An outline of the history of anthecology or pollination biology. *In* L. Real. (ed.), Pollination Biology. Academic, New York.

Bredemeijer, G. M. M. 1974. Peroxidase activity and peroxidase isoenzyme composition in self-pollinated, cross-pollinated, and unpollinated styles of *Nicotiana alata*. Acta Bot. Neerl. **23**: 149-157.

Bredemeijer, G. M. M. 1979. The distribution of peroxidase isoenzymes, chlorogenic acid oxidase, and glucose-6-phosphate dehydrogenase in transmitting tissue and cortex of *Nicotiana alatastyles*. Acta Bot. Neerl. **28**: 197-203.

Dubray, G. and G. Bezard. 1982. A highly sensitive periodic acid -silver stain for 1,2-diol groups of glycoproteins and polysaccharides in polyacrylamide gels. Analyt. Biochem. **199**: 325-329.

Faegri, K. and L. Van Der Pijl. 1979. The principles of pollination ecology. Pergamon, Oxford.

Ferrari, T. E., N. Bruns, and D. H. Wallace. 1981. Isolation of a plant glycoprotein involved with control of intercellular recognition. Plant Physiol. **67**: 270-277.

Gaude, T. and C. Dumas. 1986. Organization of stigma surface components in *Brassica* J. Cell. Sci. **82**: 203-216.

Galil, J. and M. Zeroni. 1967. On the pollination of *Ziziphus spina-christi* (L.) Willd in Israel. Isr. J. Bot. **16**: 71-77.

Grover, A. and S. K. Sinha. 1985. Senescence of detached leaves in pigeon pea and chick pea: Regulation by developing pods. Physiol. Plant. **65**: 503-507.

Gunatilleke, C. V. S. and I. A. U. N. Gunatilleke. 1984. Some observations on the reproductive biology of three species of *Cornus* (Cornaceae). J. Arnold Arbor. **65**: 419-427.

Harvey, C. F. and J. E. Braggins. 1985. Reproduction in the New Zealand taxa of *Pomaderris* Labill. (Rhamnaceae). New Zealand J. Bot. **23**: 151-156.

Heslop-Harrison, Y. and K. R. Shivanna. 1977. The receptive surface of the Angiosperm stigma. Ann. Bot. **41**: 1233-1258.

Hinata, K., T. Nishio, and J. Kimura. 1982. Comparative studies. on S-glycoproteins purified from different S-genotypes in self-incompatible *Brassica* species II. Immunological specificities. Genetics **100**: 649-657.

Jefferies, C. J. and P. Brain. 1984. A mathematical model of pollen-tube penetration in apple styles. Planta **160**: 52-58.

Johnson-Flanagan, A. M. and J. N. Owens. 1985. Peroxidase activity in relation to superization and respiration in white spruce (*Picea glauca* (Moench) Yoss) seedling roots. Plant Physiol. **79:** 103-107.

Larsen, K. 1977. Self-incompatibility in *Beta vulgaris* L. I. Four gametophytic comlementary S-loci in sugar beet. Hereditas **85**: 227-248.

Lloyd, D. G. and C. J. Webb. 1986. The avoidance of interference between the presentation of pollen and stigmas in angio-

sperms I. Dichogamy. New Zealand J. Bot. **24**: 135-162.

Lundqvist, A. 1964. The nature of the two-loci incompatibility in grasses. IV. Interaction between the loci in relation to pseudo-compatibility in *Festuca pratensis* Huds. Hereditas **52**: 221-234.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol. **34**: 125-128.

Mattsson, O., R. B. Knox, J. Heslop-Harrison, and Y. Heslop -Harrison. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature **247**: 298-300.

Merril, C. R., D. Goldman, and M. L. Van Keuren. 1983. Silver staining methods for polyacrylamide gel electrophoresis. Methods Enzymol. **96**: 230-239.

Murray, B. G. 1986. Floral biology and self-incompatibility in Linum. Bot. Gaz. **147**: 327-333.

Nasrallah, J. B. 1989. Molecular genetics of self-incompatibility in *Brassica*. *In* E. Lord and G. Bernier (eds.), From floral induction to pollination. American Society of Plant Physiologists, Maryland, USA, pp. 156-164.

Osterbye, U. 1975. Self-incompatibility in *Ranunculus acris* L. Genetics, interpretation and evolutionary aspects. Hereditas **80**: 91-112.

Roberts, I. N., D. A. Stead, D. J. Ockendon, and H. G. Dickinson. 1979. A glycoprotein associated with the acquisition of the self-incompatibility system by maturing stigma of *Brassica oleracea*. Planta **146**: 179-183.

Shivanna, K. R., Y. Heslop-Harrison, and J. Heslop-Harrison. 1978. The pollen-stigma interaction: bud pollination in the Cruciferae. Acta Bot. Neerl. **27**: 107-109.

Takayama, S., A. Isogai, C. Tsukamoto, Y. Ueda, K. Hinata, K. Okazaki, and A. Suzuki. 1986. Isolation and some characterization of S-locus-specific glycoproteins associated with self -incompatibility in *Brassica campestris*. Agric. Biol. Chem. **50**: 1365-1367.

Van Went, J. L. and M. T. M. Willemse. 1984. Fertilization. *In* B. M. Johri (ed.), Embryology of Angiosperms. Springer-Verlag, New York.

Vithanage, H. I. M. V. 1984. Pollen-stigma interactions: Development and cytochemistry of stigma papillae and their secretions in *Annona squamosa* L. (Annonaceae). Ann. Bot. **54**: 153-167.

Webb, C. J. and D. G. Lloyd. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Hercogamy. New Zealand J. Bot. **24**: 163-178.

Ziziphus mucronata subsp. *mucronata* (鼠李科) 的開花：開花期、授粉和蛋白質合成

P. C. Zietsman[1] and F. C. Botha[2]

[1]National Museum, P. O. Box 266, Bloemfontein, Republic of South Africa

[2]Department of Botany, University of the Orange Free State, Bloemfontein, 9300, Republic of South Africa

Ziziphus mucronata 自交不親和單型花是雌雄蕊異時成熟且雄蕊先熟的。同花雌雄蕊異熟是完全的，而且花可進一步被視爲異雌雄蕊異熟的。以過氧化氫、癒瘡木酚(guaiacol)和過氧化酶活性當作柱頭有感受性的指標，做爲開花期中形態上的評估是不適當的。使用放射性標記測定柱頭組織蛋白質合成速率，可做爲柱頭是否有感受性的一種敏感、容易且可信的方法。有六種基因產物只在有感受性的柱頭組織中出現，這些被誘發的多胜肽爲不含 D-甘露糖(D-mannose)或 D-葡萄糖(D-glucose)的醣蛋白。有四種分子量分別是 63.4, 57.7, 54.4 和 40 kD 的醣多胜肽存在於柱頭組織的所有時期。