BIOLOGY OF *STRIGA HERMONTHICA*

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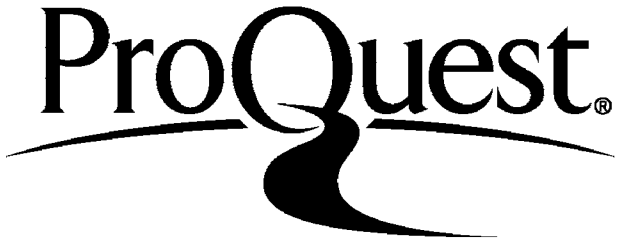

ABSTRACT

Striga hermonthica (Scrophulariaceae: Rhinanthoideae) is a parasitic weed on sorghum and millet in Africa. It has not been possible, so far, to breed varieties of these crops immune to striga. Variation in many floral and vegetative features and in seed coat ornamentation indicates a diversity of genotypes within populations of S. hermonthica.

The colour and structure of S. hermonthica flowers and their production of nectar are adaptations for cross-pollination by "long-tongued" insects like butterflies and moths; the floral biology of the species promotes outbreeding. Evidence for a gametophytic system of self-incompatibility was obtained for plants from Sudan using fluorescence microscopy to study the behaviour of pollen on stigmas. Plants grown from seed samples of S. hermonthica populations from Ethiopia, Sudan, Nigeria, Niger, Upper Volta, Mali, Ghana and Ghambia were tested and were also found to be self-incompatible. Crosses made between plants from different countries and from different host species were found to be compatible and set seed. Because there are no physiological barriers to gene exchange, variability is maintained throughout the range of the species by obligate outbreeding.

There is much variation in the response of a host variety when tested, not only against different samples of S. hermonthica seed from different localities, but between repetitions using the same seed sample. This variability occurs in controlled conditions and in sick plots, as well as in the field. It indicates that bulk samples of striga seed are also variable for genes determining the host-parasite relationship. This brings

into question the effectiveness of the standard techniques used for screening crops for resistance to the parasite. Evidence for the breakdown of resistance to striga was obtained from field observations in Sudan.

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CHAPTER 1

INTRODUCTION

ECONOMIC SIGNIFICANCE OF *STRIGA HERMONTHICA*

Striga (Scrophulariaceae: Rhinanthoideae) is a genus of parasitic flowering plants confined to the warmer regions of the old world. The Latin word "striga" means both "witch" and a row of grain that has been cut down: both meanings apply quite well to effect of striga on its host plant (Musselman, 1980). The most important species economically are *Striga hermonthica* (Del.) Benth., *S. asiatica* (L.) Ktze. and *S. gesnerioides* (Willd.) Vatke. All three species are common in Sudan (Andrews, 1956), but *S. hermonthica* is the most common and the most important economically. It attacks most, if not all, the graminaceous crops, specially sorghum, pearl millet, maize and rice (Doggett, 1965; Hosmani, 1978; McGrath, Shaw, Jansen, Lipscomb, Miller and Ennis, 1957). The effect varies from an insignificant to a serious reduction of yield depending on the level of infestation (Doggett, 1970). Since sorghum contributes two-thirds of the small farmers' everyday diet in Sudan (El Heweris, 1979), this shows the extent to which striga is affecting the economics of the country.

Striga hermonthica exists as two main strains in Sudan: one associated with sorghum and the other associated with the millet *Pennisetum americanum* (Wilson-Jones, 1955). The sorghum strain is predominantly in eastern Sudan where sorghum is the main crop and where the soil type and the rainfall are favourable for growing sorghum. The millet strain is mainly found in western Sudan where the light sandy soil and the low rainfall allow only the drought-resistant millet to be grown. Bebawi (1981) provided evidence for the existence of intraspecific variants of *S. hermonthica* by testing eleven seed collections from different

parts of Sudan against 27 sorghum cultivars, obtaining a wide range of responses.

The recent attention that has been paid to the striga problem can be attributed, according to Musselman (1980), to three main reasons. Firstly, there was the accidental introduction of Striga asiatica to the Carolinas of the USA. This has lead to wider publicity and more interest in the problem due to the threat it puts to the production of maize, which is a major crop in the United States. Secondly, there was the disastrous drought of 1968-73 in sub-saharan Africa which led research workers to look into every possible way of increasing food production. Finally, several reviews on parasitic flowering plants have appeared in the last decade (e.g. Hosmani, 1978; Kuijt, 1969) which emphasized the need for controlling the devastating effect they have on crops yield.

THE BIOLOGY OF STRIGA HERMONTHICA

It is fundamentally important, if successful control programs are to be developed, that the life-cycle of a parasite should be thoroughly understood. Much progress has been made in this field recently for Striga species.

A single plant of Striga hermonthica can produce up to 500,000 black, minute seeds which remain viable in the soil for more than 20 years (McGrath et al., 1957). After release, seeds have to undergo an after-ripening period before they can germinate. The length of this varies according to different authors, but it is between one month and two years. Vallance (1950) reported that the germinability increased as the seed aged.

After the seeds have completed their after-ripening period they have to be moisture-treated for a certain length of time at

an appropriate temperature before they can respond to germination stimulants. The effectiveness of the moisture-treatment, or the "pretreatment" as it was termed by Brown and Edward (1944), depends on the after-ripening stage of the seed, i.e. its age. The optimum pretreatment temperature for S. hermonthica was found to be 22° C (Vallance, 1950). Parker, Hitchcock and Ramaiah (1977) found that the higher the temperature (23°-33° C) the shorter the time needed for pretreatment (from 14 down to 7 days).

Pretreated seeds require a stimulant to initiate germination, normally a root exudate from a host plant . Other non-host plants have been found to stimulate germination, like cotton and sunflower (McRath et al., 1957). A few chemical compounds are known to induce germination of striga seed. These include ethylene (Egley and Dale, 1970), kinetin, coumarin derivatives and some other substituted purines (Worsham, Klingman and Moreland, 1962). Old seed were reported to germinate without the need for a stimulant (Vallance, 1951).

After germination, the tip of the radicle develops a haustorium which attaches to the host's root and makes a vascular bridge. Certain stimulants are needed for this stage to be accomplished. Okonkow (1966) reported that S. hermonthica growing in a sterile culture did not produce any haustoria. Gum tragacanth has been reported to stimulate haustoria formation in germinating striga seeds (Musselman, 1980). It seems a stimulant is needed at each stage of S. hermonthica development: germination, haustoria initiation, penetration and physiological compatibility (Musselman, 1980). A host-plant proper is the one which is capable of providing the whole set of stimuli.

After successful attachment to the host's root, penetration seems to be achieved through enzymic action (Ba and Kahlem, 1979). A vascular bridge is then formed to connect the conducting tissues of the parasite to the host's. The xylem connection is

well documented but there is no report of a phloem connection in the haustorium region. Transport of elaborate food material from host to parasite has been thought to take place through the xylem (Okonkβw, 1964). A separate pathway for organic material has been reported by Roger and Nelson (1962) and confirmed, using fluorescence microscopy, by Safa and Jones (1983).

After successful establishment has been achieved the parasite's seedling remains underground for a period of time varying in length from 7 to 49 days, or even longer depending on the host and the environmental conditions. During this period, striga leads a typically holo-parasitic mode of life since it lacks chlorophyll and hence depends completely on the host for nourishment.

Once emerged above soil-level the striga seedling turns green and starts leading a semi-parasitic life. The degree of striga's dependence on its host is a matter of controversy. Using radioactive carbon dioxide, the transport of elaborate photosynthates from host to parasite has been reported by several authors (e.g. Okonkβw, 1966; Ismail and Obied, 1976). Williams (1961) observed that albino S. hermonthica continued to grow for some weeks. On a field trip in Sudan with Dr. Lytton Musselman in 1982, we found an albino S. hermonthica which was flowering. This shows that striga can stay dependant on its host throughout its life. Striga was reported to have a toxic effect on its host (Kust, 1966). From the above mentioned facts it seems unlikely that striga is dependant on its host for water and solutes only, after it emerges above soil level. A more complicated relationship seems to exist between host and parasite involving nitrogen-metabolism and growth-regulation (Drennan and El Heweris, 1979).

The time from emergence to flowering depends on the host-species and the environmental conditions.

CONTROL OF *STRIGA HERMONTHICA* IN THE FIELD

Most of the effort to control *Striga* has been concentrated upon the germination stage. Many biological and chemical means have been employed to induce suicidal germination of the seed in the soil. These include catch-crops, trap-crops and the application of germination stimulants.

Catch-crops are proper host plants that can stimulate and support the striga establishment. These are ploughed under to stop the parasite reaching the flowering stage (Parker, 1965). Trap-crops are those plants which produce a germination stimulant but cannot support the establishment of the parasite.

The chemical nature of the germination stimulant from cotton plants has been characterized from root extracts (Cook, Wichard, Wall, Egley, Coggan, Luhan and McPhail, 1972); it has been named "strigol". Strigol was synthesized by Heather, Mittel and Sih (1974). Many strigol analogues, named "GR-compounds", like GR7, GR24, GR28, and GR45, were synthesized by Johnson, Rosebery and Parker (1976). The activity of different GR-compounds has been studied under different environmental conditions in Sudan (Babiker and Hamdoun, 1982 and 1983). Ethylene gas was found to be a very powerful germination stimulant if injected into a soil infested with striga seed. Much evaluation of the effectiveness of ethylene in controlling *S. asiatica* has been done in the Carolinas, USA (Eplee, 1975; Egley *et al.*, 1970).

Various herbicides have been applied and favourable results have been obtained using 2,4-D and related compounds (Eplee and Langston, 1976, Wilson-Jones, 1953). Other herbicides which were reported to be effective include chlorofenac (Robinson, 1961), trifluralin and MCPA (Kassasian, 1971).

Nitrogenous fertilizers were reported to offer a favourable degree of striga suppression (Andrews, 1947; Agabawi and Younis, 1965), but the exact mechanism of suppression and its time of action is not known (El Heweris, 1979).

Hand-weeding was reported to be effective in increasing crop yield (Doggett, 1970), but this method is expensive and not practical in heavily infested areas.

A farmer of one of the large semi-mechanized farms of the Gedarif area of Sudan noticed that when the established sorghum crop (50 cm tall), which was infested with striga, was shallow-ploughed the yield was increased. He also observed that if the process was repeated for three to five seasons, striga was reduced to a minor problem in the fields (personal communication, 1982). This shallow-ploughing seems to break the striga-sorghum union of those roots near the surface (which seem to be the ones mainly attacked by striga) and hence destroys most of the striga plants before they reach the flowering stage. The sorghum would naturally also be affected due to damage to the upper part of the root system, but the this would soon be regenerated.

The methods of control mentioned above proved to be very expensive and beyond the reach of small farmers in developing countries. So most of the efforts have been concentrated, in the last few years, on developing crop cultivars resistant to striga attack.

BREEDING FOR RESISTANCE TO *STRIGA HERMONTHICA*

Doggett (1970) recognized two forms of resistance in cultivars resistant to striga attack: 1) low production of germination stimulant and, 2) resistance based on failure of striga to establish on host roots due to mechanical or physiological barriers. The first type of resistance was found in

sorghum varieties like "Framida", and the second type was recognized in "Dobbs" (Doggett, 1970).

To identify each type of resistance, several methods of screening have been developed. The best method of assaying the production of germination stimulant is the double-pot technique developed by Parker et al. (1977). This method is supplemented with the pot-technique to identify other forms of resistance. These methods are dealt with in more detail in Appendices 3 and 4.

The results obtained using the different techniques have been inconsistent and quite variable even under controlled conditions*. Nothing seems to be wrong with the techniques themselves, so the inconsistency should either be something to do with the crop varieties or with the parasite itself. Since the crop varieties have been selected for morphological and physiological uniformity, the variation in results could be due to features intrinsic to the striga samples used in testing. So far little attention has been paid to the genetics of the parasite in attempts to improve the crop species: I have been unable to find any publications concerned with the genetics of S. hermonthica itself, though its variability (or lack of it) has implications to all work with striga.

The work presented in this thesis has been devoted to a study of the variation in S. hermonthica populations in an attempt to determine how far the variability of the parasite may be affecting the progress of striga research. To this end, morphological and physiological variation has been examined and the breeding-system, which contributes to that variation, has also been studied.

* Verbal reports by C. Parker, K.V. Ramaiah, F.F. Bebawi and L.J. Musselman. (Also refer to pp. 66 and 68).

CHAPTER 2

MORPHOLOGICAL VARIATION IN STRIGA HERMONTHICA POPULATIONS

INTRODUCTION

The wide range of variation in Striga hermonthica has prompted some workers to attemp to recognize more than one species within it. Bentham 1868 has recorded two species: S. hermonthica and S. senegalensis, with the latter "very closely resembling S. hermonthica and is probably only a small-flowered form of it" (in Oliver 1868).

Wilson-Jones, who worked on agronomic problems associated with striga attack on crop plants in Sudan, wrote in a letter to Milne-Redhead of the Royal Botanic Gardens at Kew, in 1954 "...my strains more or less coincide with the hermonthica-senegalensis separation..... My belief is 1) that flower size is very variable, partly according to host, and 2) flower colour seems variable, but partly according to soil type" (Wilson-Jones, 1954).

Variation in floral features other than those mentioned above have been noticed by Musselman, Nickrent, Mansfield and Ogborn (1979) on a visit to Nigeria. They noticed that there is much variation in the shape of the upper and lower lips of the corolla, and in the colour of the throat of the corolla-tube.

In this investigation I am reporting my studies on variation in some obvious and less obvious features both of natural S. hermonthica populations in the field (in Sudan) and among plants grown from seed samples of striga seed from various African countries under glasshouse conditions in England.

MATERIALS AND METHODS

Observations were made on plants growing in the well-established test plots of the Agricultural Research Farm at Wad Medani, Sudan in 1982 .

Some seed samples of Striga hermonthica used in this study were collected in Sudan, in 1980; others were obtained from the seed stock held by the Weed Research Organization, Oxford, UK which were collected from different parts of Africa (Table 2.1). Plants were raised in pots in a glasshouse at Egham, Surrey, UK using the method described by Parker and Reid (1979). Sorghum cultivar CK60B and the millet cultivar Ex-Bornu were used as host plants to support striga establishment. The temperature in the glasshouse was kept above 25°C all the time and the plants were provided with supplementary light for 12 hours daily, using 400W mercury lamps suspended 1.7 metres above the pots.

Table 2.1 Locality, date of collection and host species for the seed samples of Striga hermonthica grown in the glasshouse at Royal Holloway College, Egham, Surrey, UK.

country	date of collection	host
Ethiopia	1978	sorghum
Sudan (Shambat)	1980	"
Nigeria	1977	"
Niger	1979	"
Mali	1977	"
Upper Volta	1979	"
Ghana	1977	"
Ghambia	1979	millet

Table 2.2 Origin and host for Striga hermonthica seed collections examined using the Scanning Electron Microscope.

Host plant species	Location and year of collection	seed source
Pennisetum americanum	Gambia 1979	WRO
P. americanum	Sudan 1978	WRO
Sorghum bicolor	Nigeria 1977	WRO
S. bicolor	Gambia 1979	WRO
S. bicolor	Sudan, Shambat 1980	SS
S. bicolor	Sudan, Shambat 1979	KUFA
S. bicolor	Sudan, Abu Naama 1980	KUFA
S. bicolor	Sudan, Nesheshiba 1980	SS
S. sudanense	Sudan, Nesheshiba 1980	SS

Key to sources of seeds:

KUFA: Khartoum University, Faculty of Agriculture (bulk collection),

SS: collected by the author from single capsules,

WRO: Weed Research Organization, Oxford, UK (bulk collection).

The following ten characters were studied: length of the largest leaf on the plant and its width, the length and width of the corolla of the lower-most open flower on an inflorescence and its calyx length, corolla colour and colour uniformity, corolla margin (entire or dentate), the position of the stigma relative to the calyx in the lower-most open flower and the internode length between flower nodes.

Plants for study were chosen at random: 120 from the test-plots in Wad Medani and 12 plants from each of the seed samples detailed in Table 2.1.

For studying seed-coat ornamentation seeds of S. hermonthica were obtained from different localities as detailed in Table 2.2. The seeds brought from Sudan were collected either from one capsule, "single plants" or as a mixture, "bulk collection", gathered from different plants growing on a single host species.

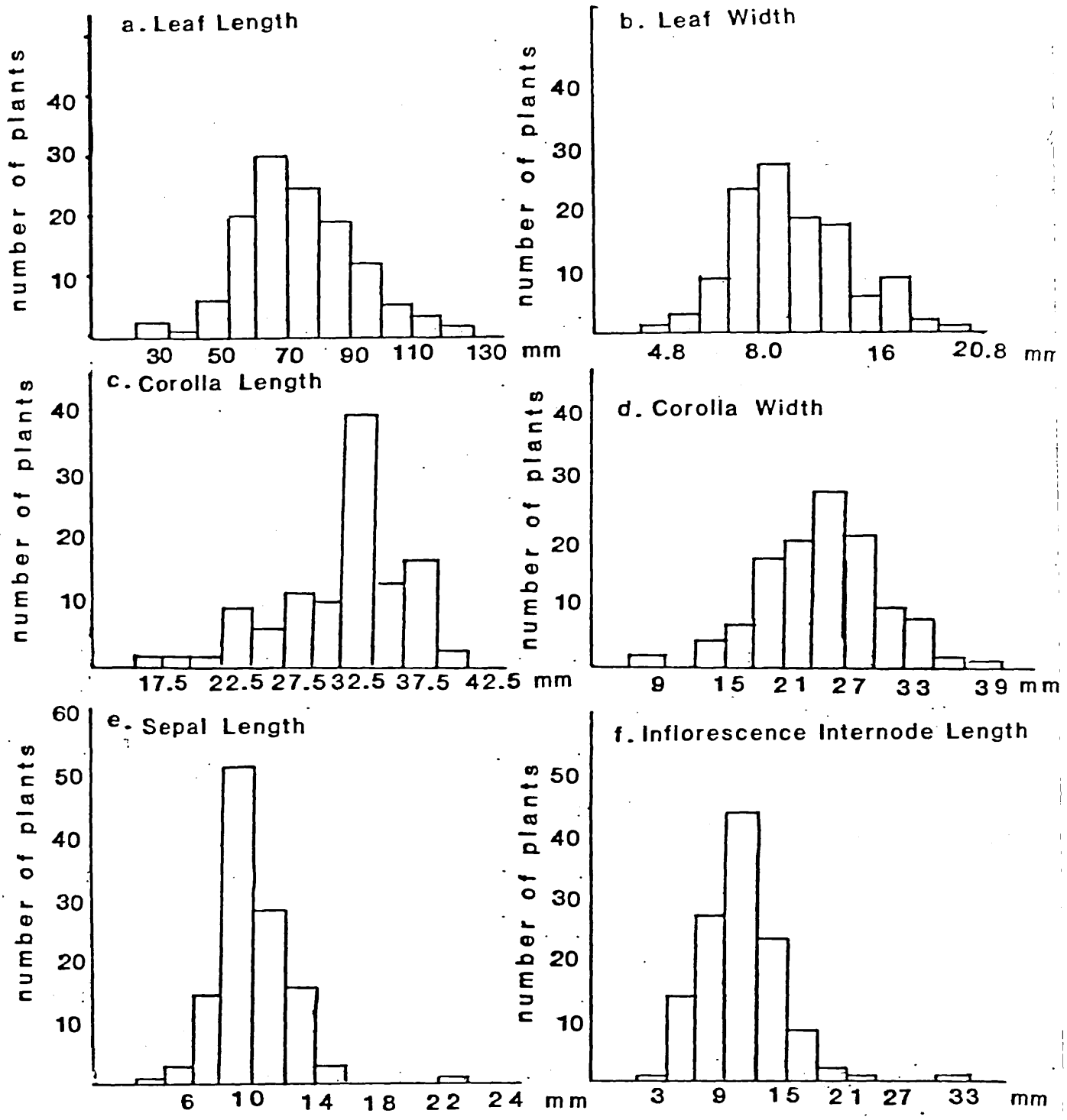
Seeds were mounted on stubs using double-sided adhesive Sellotape and coated with 45 nm of gold-palladium in a Polaron Cool sputter-coater and examined in a JEOL scanning electron microscope. Care was taken to record the surface features only on perfect, fully-developed seeds.

I am following Musselman and Mann (1976) in using the terminology of Chuang and Heckard (1972) for describing seed-coat ornamentation.

RESULTS

The results of the morphological investigation are presented as follows. Firstly the data obtained from the 120 plants growing at Wad Medani, Sudan are given in Appendix 1, and the equivalent data from the 8 seed samples raised at Egham, England are given in Appendix 2.

Fig. 2.1 Histograms of the six quantitative characters (Appendix 1) measured from a sample of 120 S. hermonthica plants collected from a sick-plot at Wad Medani, Sudan.



The results for each of the ten morphological characters studied are then reviewed, with reference to the analysis of variance given in Table 2.3 and the visual indication of variability provided by the histograms (Fig. 2.1).

The degree of correlation between the ten characters is examined and the data for this is presented in Table 2.4, for the Wad Medani population, and in Table 2.5, for the seed samples from Africa grown in England.

Table 2.4 Correlation matrix of 10 morphological characters studied on a natural population of Striga hermonthica growing in Sudan. (see Appendix 1 for the data).

ll	1.00					DF= 118				
lw	0.58	1.00				Critical r Values				
cl	0.35	0.20	1.00			P: (.05)	r= .18			
cw	0.33	0.23	0.72	1.00		(.01)	r= .23			
cc	0.11	0.05	0.09	0.02	1.00	(.001)	r= .29			
cm	-0.16	-0.03	-0.10	-0.10	-0.15	1.00				
ccu	0.02	0.10	0.14	0.03	0.05	-0.08	1.00			
sl	0.55	0.32	0.37	0.32	0.11	-0.10	0.11	1.00		
ssp	-0.04	0.10	-0.34	-0.28	-0.05	0.17	-0.03	-0.02	1.0	
iil.	0.27	o.19	0.41	0.33	-0.01	-0.26	0.02	0.21	-0.43	1.00
	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil.

(ll : leaf length, lw : leaf width, cl : corolla length, cw : corolla width, cc : corolla colour, cm : corolla margin, ccu: corolla colour uniformity, sl : sepal length , ssp: position of stigma relative to sepals iil: inflorescence internode length).

Table 2.5 Correlation matrix of 10 morphological characters studied on a wide sample of Striga hermonthica grown in a glass-house in UK (see Table 2.1 for details of the sample and Appendix 2 for the data).

11	1.00					DF= 94				
lw	0.09	1.00				Critical r Values				
cl	-0.02	0.06	1.00			P: (.05)	r= .20			
cw	-0.07	0.06	0.44	1.00		(.01)	r= .26			
cc	-0.05	0.05	0.012	0.14	1.00	(.001)	r= .33			
cm	0.15	0.10	0.12	0.23	-0.12	1.00				
ccu	0.21	0.02	0.13	0.03	-0.09	0.06	1.00			
sl	0.08	-0.11	0.07	-0.03	-0.02	-0.08	0.12	1.00		
ssp	0.07	0.05	-0.11	-0.27	-0.14	-0.08	0.06	0.09	01.00	
iil.	0.14	-0.11	0.02	-0.10	0.05	0.02	0.12	0.01	0.05	1.00
	11	lw	cl	cw	cc	cm	ccu	sl	ssp	iil.

(Abbreviations as in Table 2.4)

Leaf Length and Width:

The leaves of Striga hermonthica are quite variable, even on the same plant. The leaves at the upper and lower extremeties of the stem are small compared to the middle ones. The measurements presented in Appendix 1 and Appendix 2 show the length and width of the largest leaf on the stem. The distribution of both length and width is normal (Fig. 2.1a and 1b) and a wide range of variation in both is quite evident from Fig. 2.2. The correlation between leaf-length and width is small in Table 2.5, but very significant in Tables 2.4. Analyzing the data collected from the glass-house plants using the Analysis of Variance methods, there seems to be a significant difference between the different populations of S. hermonthica with regard to their leaf dimensions (Table 2.3.1 and 2.3.2).

Table 2.3 Analysis of Variance for 6 of the quantitative characters measured for the striga plants grown from African seed samples.

2.3.1: Leaf Length

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	78.1	80.9	82.8	89.3	107.2	80.3	81.3	79.7

Source	DF	SS	MS	F
Total	95	29337.8		
Sample	7	7716.0	1102.3	4.5 **
Error	88	21621.8	245.7	

L.S.D (P=0.05)= 16.67
(P=0.01)= 22.06

2.3.2: Leaf Width

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	10.8	14	7.6	7.3	9.8	6.8	7.1	7.3

Source	DF	SS	MS	F
Total	95	737.3		
Sample	7	537.0	76.6	33.7 **
Error	88	200.3	2.3	

L.S.D. (P=0.05)= 1.61
(P=0.05)= 2.13

2.3.3: Corolla Length

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	24.1	24.4	24.7	23.5	23.3	25.8	22.3	21.5

Source	DF	SS	MS	F
Total	95	2283.5		
Sample	7	157.2	22.5	0.9 NS
Error	88	2126.3	24.2	

2.3.4: Corolla Width

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	16.8	21.4	21.2	19.8	17.2	20.7	17.9	18.8

Source	DF	SS	MS	F
Total	95	1711.0		
Sample	7	284.4	40.6	2.5*
Error	88	1426.6	16.2	

L.S.D. (P=0.05)= 4.16
(P=0.01)= 5.51

2.3.5: Calyx-tube Length

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	11.7	11.1	11.9	11.7	10.5	12.8	10.4	11.9

Source	DF	SS	MS	F
Total	95	606.2		
Sample	7	64.8	9.3	1.5 NS
Error	88	541.4	6.2	

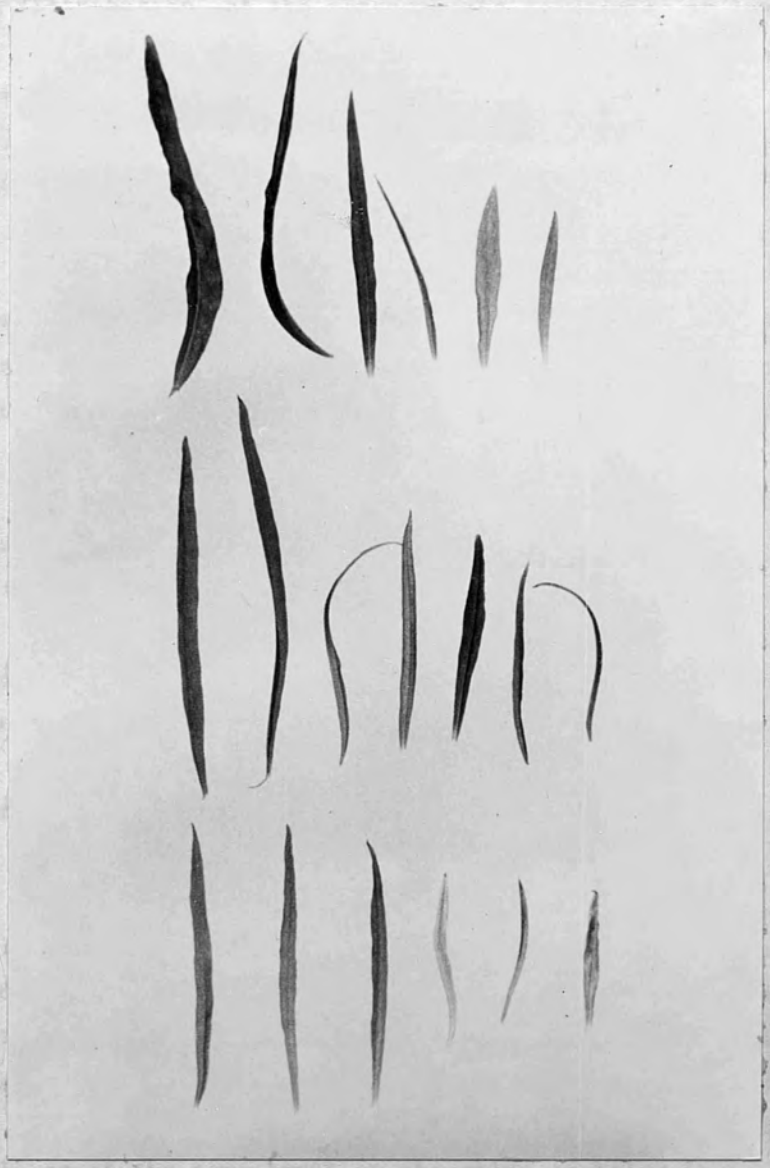
2.2.6: Inflorescence Internode Length

	ETHIOPIA	SUDAN	U.VOLTA	NIGERIA	NIGER	MALI	GHANA	GAMBIA
MEAN	10.7	8.1	11.6	11.6	13.4	11.6	12.5	8.1

Source	DF	SS	MS	F
Total	95	1719.6		
Sample	7	314.5	44.9	2.8 *
Error	88	1405.2	16.0	

L.S.D. (P= 0.05)= 4.25
(P= 0.01)= 5.65

Fig. 2.2 Leaves collected from different S. hermonthica plants of the "sorghum strain" grown from one seed sample, to show the variation in leaf dimensions. The leaves are cut from a corresponding position on every stem.



Corolla Features:

A wide range of corolla colour, from white to deep purple, were observed in all the populations of S. hermonthica studied (Fig. 2.3). For convenience, the colour was classified into three groups: pale pink (including white), medium pink and deep pink.

Corolla length and width were found to be quite variable. The distribution of the measurements seems more or less normal (Fig 2.1c and 2.1d). Analysis of Variance showed that there is no significant difference, of corolla length and corolla width (P= 0.01), between the 8 African populations studied (Table 2.3.3 and 2.3.4)

Colour uniformity of the corolla was found to be variable. Flowers with uniform colour as well as varigated (pink with white marks) were found in all the population studied. For colour uniformity flowers were classified into uniform and varigated.

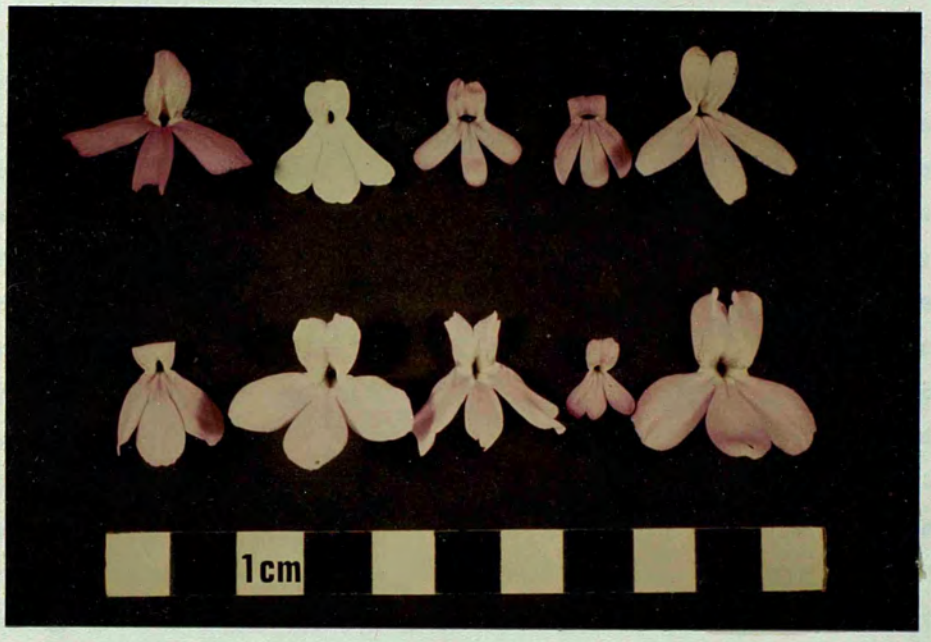
The margin of the corolla lobes was classified into entire and dentate.

The correlation co-efficient of the different corolla features was found to be insignificant in both Table 2.4 and Table 2.5.

Relative Position of the Stigma to the Calyx Tube:

The calyx tube length was found to be variable in all the populations examined. There is no significant difference between the different populations studied with regard to their calyx-tube length (Table 2.3.5). The length of the style was found to be variable as indicated by the position of the stigma relative to the calyx rim which was classified as: either above the calyx level, or at the same level as the calyx or below it.

Fig. 2.3 Flowers of different S. hermonthica plants of the "sorghum strain" grown from one seed sample collected from Sudan in 1980. They show much variation in colour, size and shape.



Inflorescence Internode Length:

The compactness of the inflorescence was found to be variable in all the populations studied. The length of the inflorescence internode (average of the six lower-most internodes) was taken as an estimate for the degree of compactness. The difference between the different populations was not found to be highly significant (Table 2.3.6).

Seed-coat Ornamentation:

The size and shape of seeds and the prominant pattern of primary ridges on the surface varied considerably in all the collections examined. I noticed that the collections of seeds from single plants were more uniform with respect to other less obvious features and I concentrated my observations upon these features. These were: the density of protuberances on primary ridges, on the secondary ridges and on the area in between them; the prominence of the rim on top of the primary ridges and the prominence of the secondary ridges. In contrast, these features varied considerably on seeds from bulk samples. Some seeds had a high density of protuberances all over the seed-coat (Fig. 2.4) while others only had them on the primary and the secondary ridges (Fig. 2.5). In a small number of seeds, protuberances were almost absent (Fig. 2.6). Indeed, a whole range of intermediates occurred between those seeds having a surface densely covered with protuberances and those which were almost smooth, in the same bulk sample (Fig. 2.7).

The rim on the top of the primary ridges varied in prominence, sometimes being broad and high (Fig. 2.4 and 2.6) sometimes being narrow and low (Fig. 2.8 and 2.9); both types are shown in Fig. 2.7. The shallow secondary ridges which run lengthwise or crosswise between the primary ridges may be prominent (Fig. 2.8 and 2.9) or obscure (Fig. 2.10 and 2.11).

Fig. 2.4 A scanning electron micrograph of part of the testa of Striga hermonthica seed gathered from a plant parasitising sorghum in a field at Shambat, Sudan. The sides of the ridges and the area betweem them are densely covered with protuberances. X 700

Fig. 2.5 A scanning electron micrograph of part of the testa of Striga hermonthica seed gathered from a second plant in the same field as Fig. 2.4. The few protuberances are confined to the sides of the ridges. X 700

Fig. 2.6 A scanning electron micrograph of part of the testa of Striga hermonthica seed gathered from a third plant in that field (Fig. 2.4 refers). The ridges and the area between are almost smooth. X 700

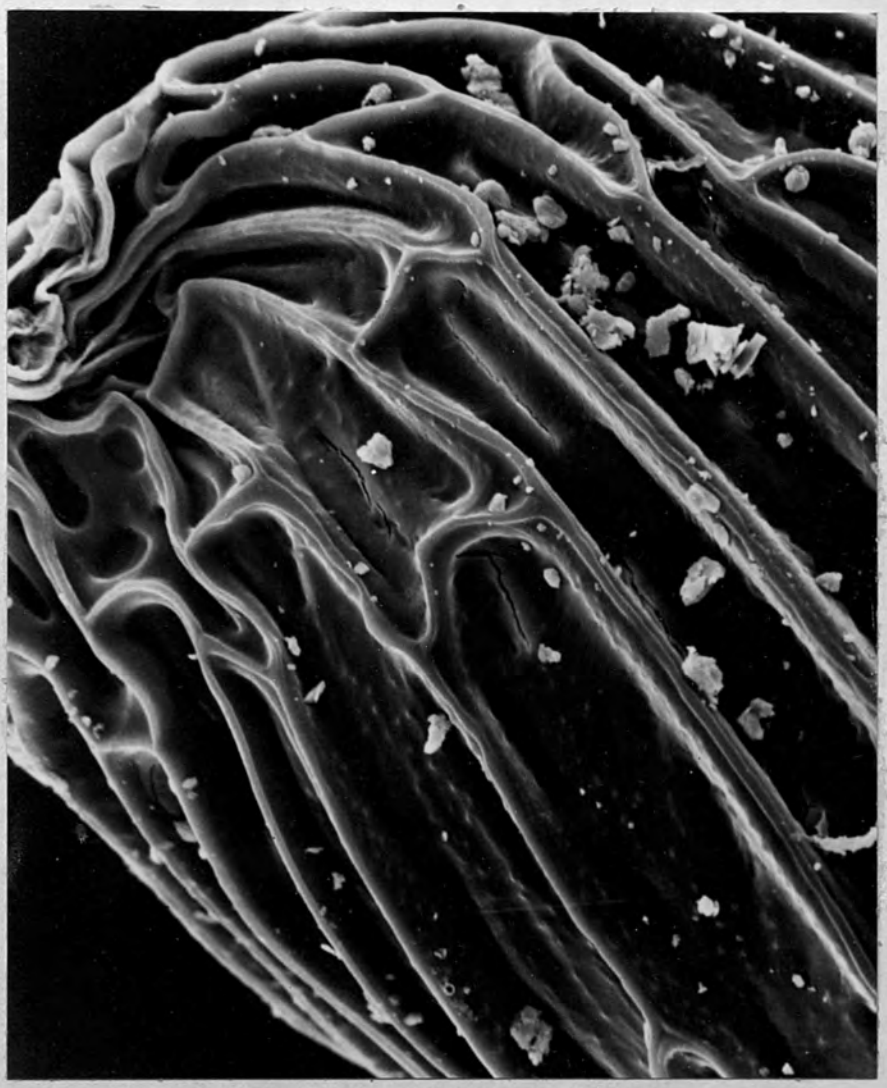


Fig. 2.7 A scanning electron micrograph of part of the testa of Striga hermonthica seeds gathered from a plant parasitising sorghum at Abu Neaama, Sudan. The surface of one seed is covered with protuberances; the other has none. X 700

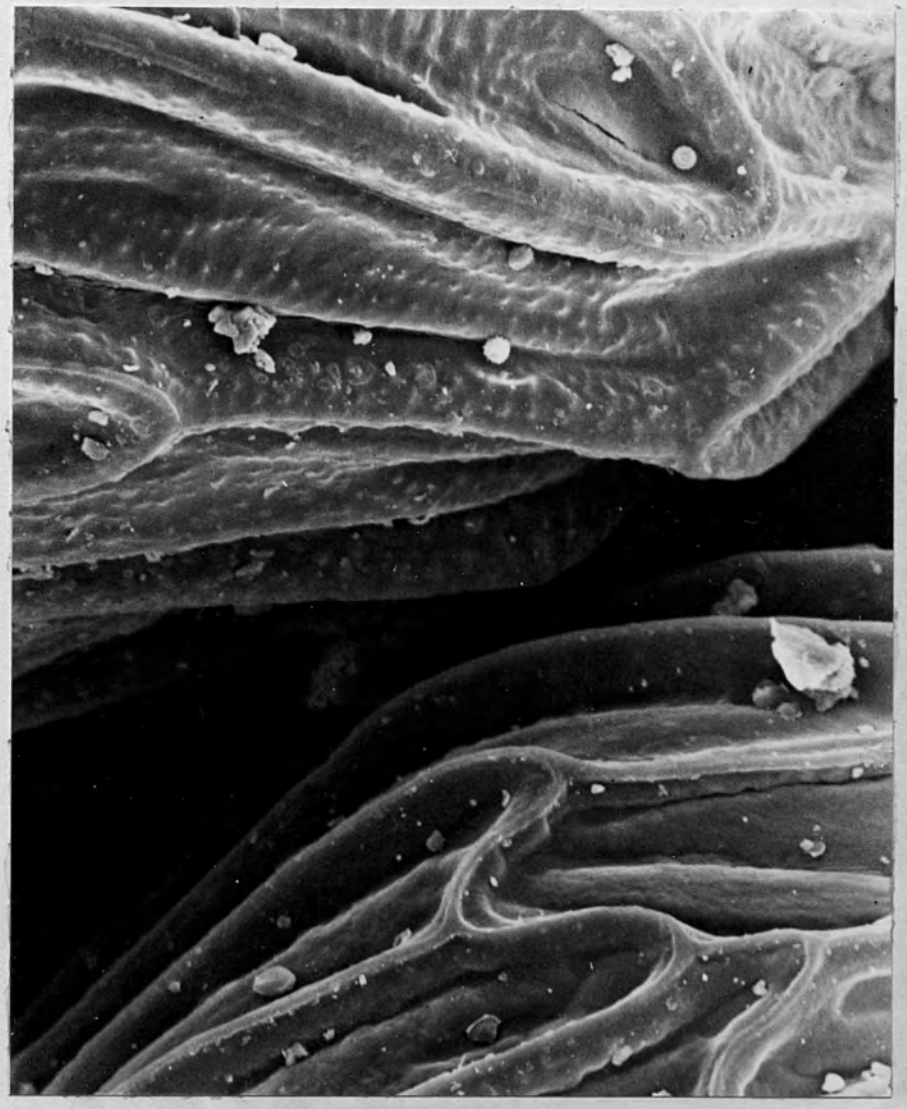


Fig. 2.8 A scanning electron micrograph of part of the testa of Striga hermonthica seed in which the protuberances are confined to the ridges which closely resembles Musselman and Parker's plate of S. gesnerioides. Seeds collected from plants parasitising sorghum at Shambat, Sudan. X 700

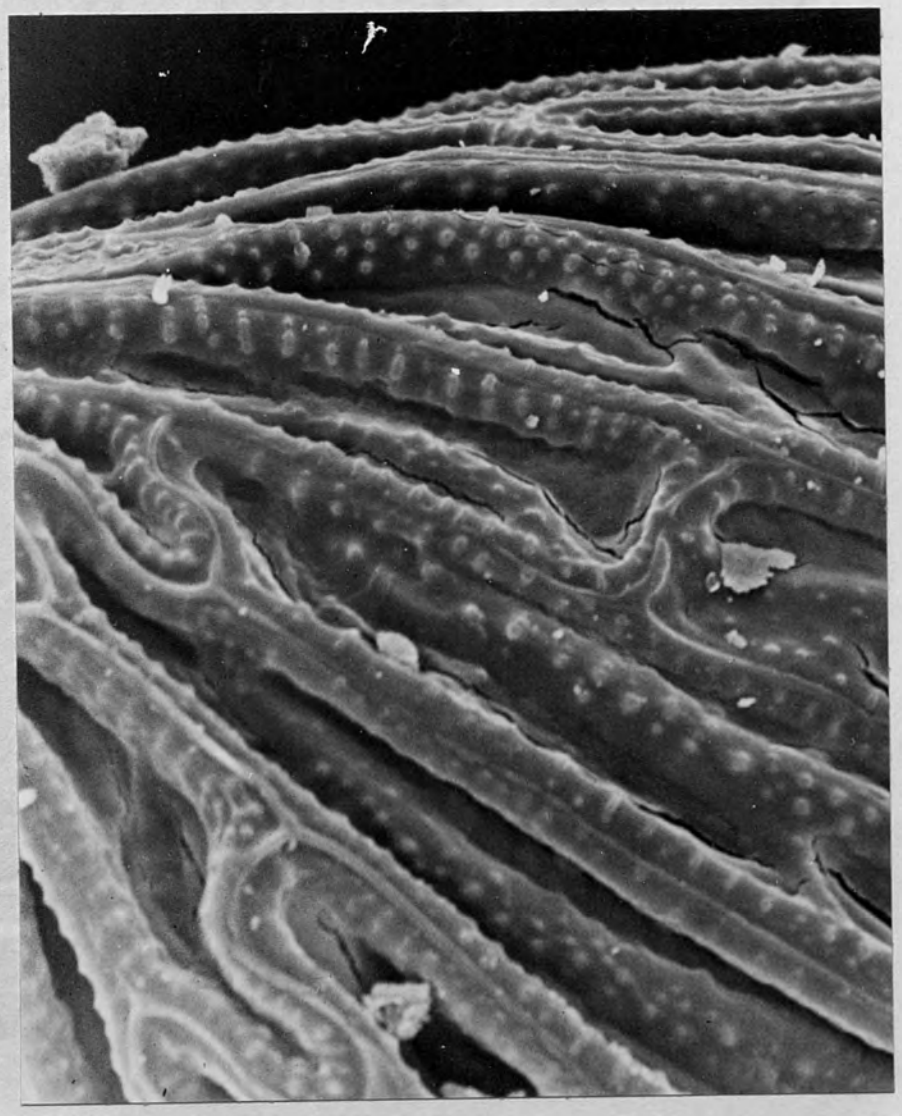


Fig. 2.9 A scanning electron micrograph of part of the testa of a Striga hermonthica seed which resembles Musselman and Parker's plates of S. asiatica. The seed is from the same collection as Fig. 2.8. X 700

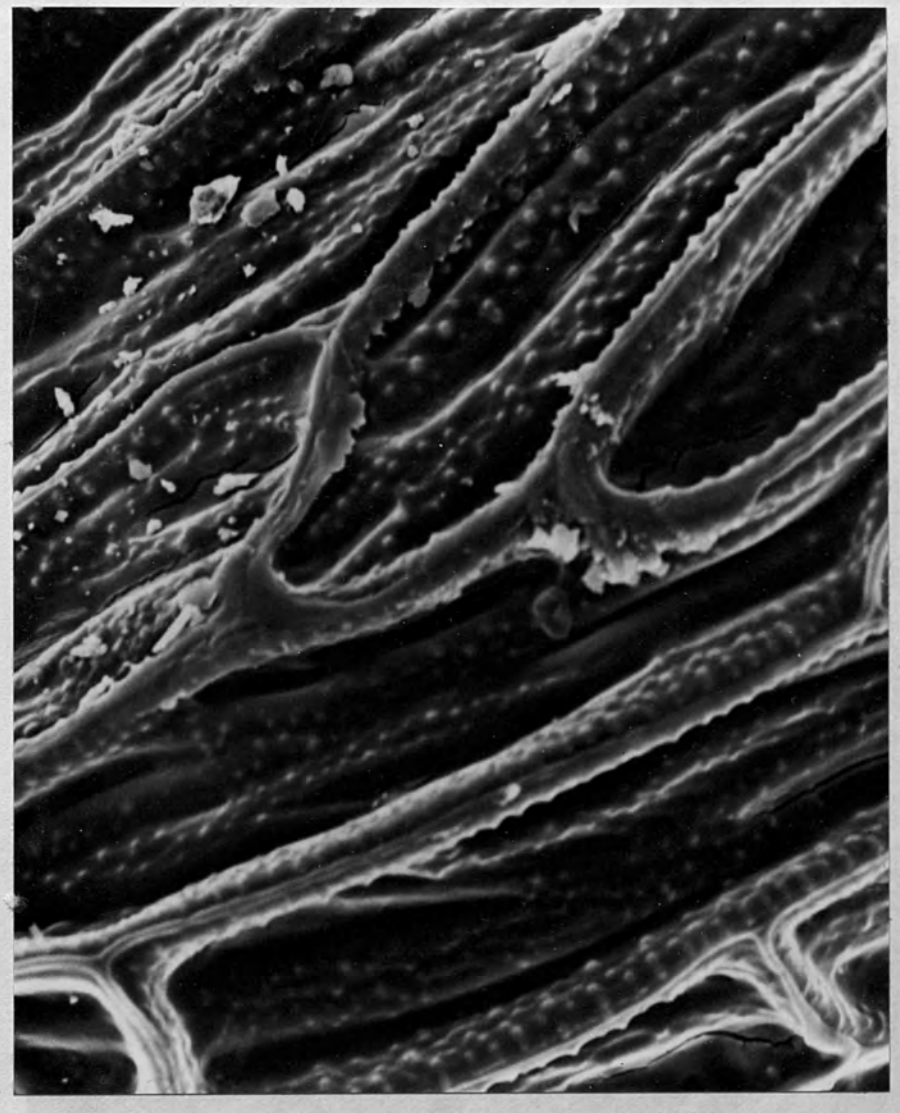
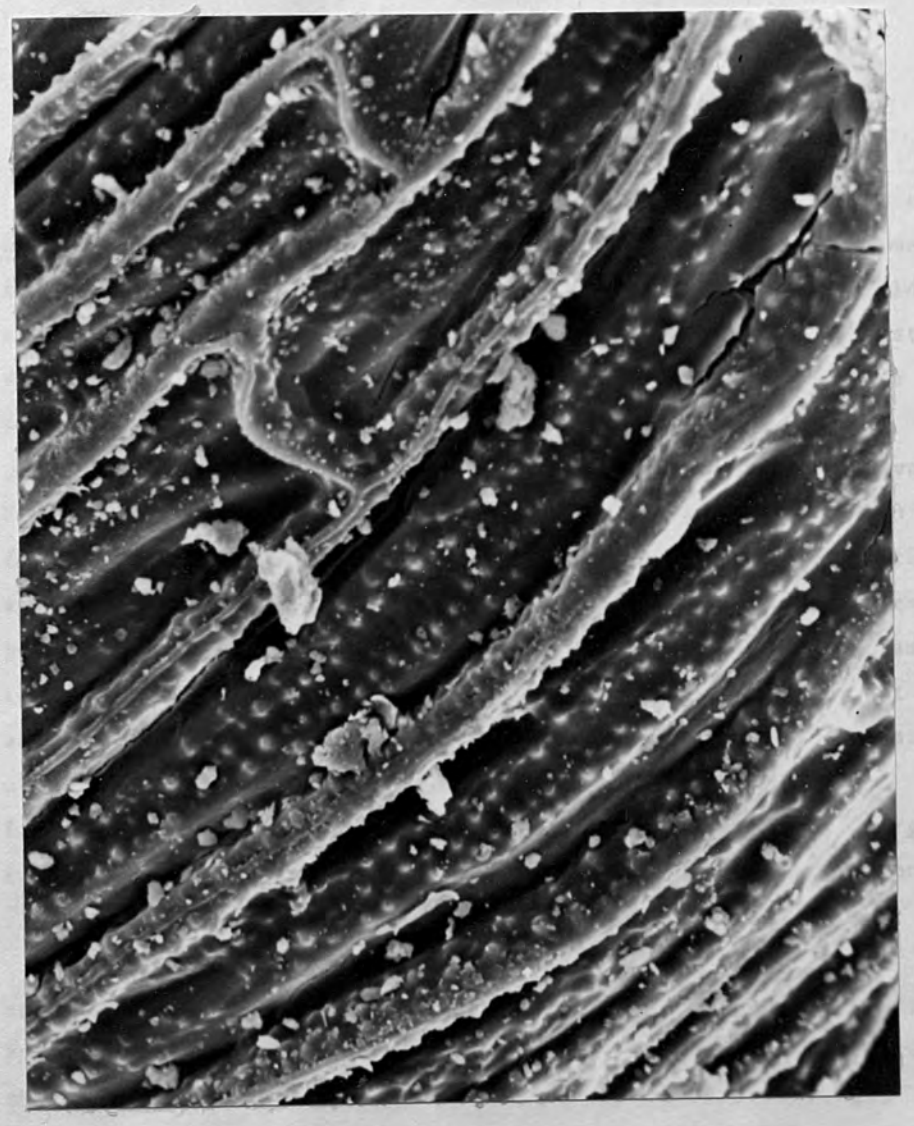


Fig. 2.10 A scanning electron micrograph of part of the testa of Striga hermonthica seed gathered from plants parasitising sorghum at Abu Geaama, Sudan. The elongated protuberances on the primary ridges and the smaller ones on the secondary ridges resemble those on Musselman and Parker's plate of S. forbesii. X 700

Fig. 2.11 A scanning electron micrograph of part of the testa of a third Striga hermonthica seed from the same collection as Fig. 2.8. The primary ridges have obscure, and the secondary ridges prominant protuberances as in Musselman and Parker's plate of S. passargei. X 700



The variation occurred irrespective of whether the collections were made from a sorghum or a pennisetum millet field. A similar range of variation was found in the collections made in different countries. Because of this wide range of variation in all the bulk samples examined I selected a few representative types for illustrative purposes to represent all the collections.

DISCUSSION

The ten morphological characters studied make it clear that S. hermonthica populations are variable in many features. Some of the features are obvious, like the leaf dimensions and the flower colour and size; the others though less obvious are still strikingly variable. Yet, despite this variation, there is no significant difference (P=0.05) between the means of any of the populations in two of the six characters measured quantitatively. Furthermore, the only highly significant difference (P=0.01) was found to be for the leaf dimensions. West African populations seems to have smaller leaves compared to the East African populations. If the difference is real, and not due to the sample tested being small, this could be explained by the fact that the seed samples collected from Western Africa was collected in areas of lower rainfall than those from Eastern Africa. Selection may favour smaller-leafed plants to be more advantageous to larger-leafed ones, since this may reduce water loss by transpiration.

The correlation test (Table 2.5) shows that virtually all combination of the characters studied are possible as indicated by the low correlation co-efficient between most of the various characters. The correlation co-efficient of corolla-length and width is high in both Tables 2.4 and 2.5 (0.72 and 0.44 respectively). The leaf-length and width seems highly correlated

in Table 2.4 (0.58), but not in Table 2.5 (0.09). The differences in the correlation co-efficients between Table 2.4 and Table 2.5 may be due to the fact that Table 2.4 is for plants growing in the same locality (in a field in Wad Medani, Sudan) while Table 2.5 is for the plants collected from 8 widely spread localities across Africa (Table 2.1). Since differences in environmental conditions may have affected the morhpology of different populations, the pooling of observations recorded in Appendix 2 may have reduced the correlation co-efficient which might normally be high (cf. Table 2.4).

The random association of most characters in individual plants can be taken to support the view that S. hermonthica exists as a large number of genotypes in all populations (Jones and Safa, 1982).

Most of the obvious variation in shape and size of S. hermonthica seeds did not seem to to be directly under genetic control. Some of the smaller and misshapen seeds appeared to be empty, having presumably been stimulated to develop by the act of pollination, but because there were a large number of ovules in each pistil, some of them might not have been fertilized. The diversity of ornamentation detail in bulked seed collections contrasted with the similar ornamentation of seed gathered from one plant indicates that the less obvious features of testa ornamentation are genetically determined.

The presence of a diversity of testa ornamentation within this one species, S. hermonthica, casts doubt upon the statement by Musselman and Parker (1981) that "surface features of seeds (of Striga species) may have taxonomic value". In my material I found ornamentation similar to those which they figure for S. gesnerioides (Fig. 2.8), S. asiatica (Fig. 2.9), S. forbesii (Fig. 2.10) and S. passargei (Fig. 2.11). This conflicts with their conclusion that "surface feature may be of value in the

study of species complexes such as the S. hermonthica / S. aspera / S. passargei group".

The occurrence of a multiplicity of biotypes among plants grown from a single seed-sample even when grown under uniform conditions, together with the discovery of a wide range of ornamentation detail not only in different populations but characterizing different individuals within populations, implies considerable genetic variability both for morphological features and for characteristics of the seed-coat in S. hermonthica. This variability could only be maintained if the species had an efficient out-breeding system. The breeding system and the floral biology of S. hermonthica is the subject of the next chapter.

[The account of seed-coat ornamentation given here was published as a paper (Jones and Safa, 1982). A copy is provided at the end of the Thesis]

CHAPTER 3

FLORAL BIOLOGY AND BREEDING SYSTEM OF *STRIGA HERMONTHICA*

INTRODUCTION

The variation I have described in the last Chapter has hindered the work of protecting the crops against *Striga hermonthica*. The origin of this variation is the subject of the investigation reported in this Chapter. Little work has been done on the breeding system of any *Striga* species. Parker, reported in Musselman, Nickrent, Mansfield and Ogborn, (1979), suggested that some populations of *S. hermonthica* may be outbred. Musselman, Parker and Dixon (1981) self-pollinated and intercrossed some *S. hermonthica* plants grown from seed of a strain specific to sorghum and noticed that only the crossed flowers set seeds.

The breeding system, because it determines the variation of this species, has an important bearing upon the plant-breeder's ability to select cereal lines reliably resistant to the parasite. We report here our investigations of the floral biology and breeding system of *Striga hermonthica*.

MATERIALS AND METHODS

Striga hermonthica plants were raised in glasshouse at Egham, Surrey, U.K., from seed collected from different parts of Africa as presented in Table 2.1, using the method of cultivation described by Parker and Reid (1979). Supplementary lighting was given to the host plant for 12 hours daily by 400W mercury vapour lamps suspended 1.7 metres above the pots. Two striga "strains" were used: one collected from plants attacking

sorghum and the other from plants attacking pennisetum millet. Sorghum cultivars CK60B and Feterita, and pennisetum cultivars Ex Bornu and Kordofani were used as hosts for striga. Single plants were grown in pots containing the appropriate strain of Striga. During the period when the observations were made the night temperature in the glasshouse was maintained at about 25° C. When flowering commenced the glasshouse was kept closed to reduce the possibility of stray visits by insects. Each flower spike emerging in a pot was treated as a separate entity for experimental purposes. Self- and cross-pollinations were made by dusting the stigmatic surfaces with parental or with foreign pollen using a brush and by carefully placing parental or foreign pollen on either side of the dorsiventrally-flattened stigma using a needle. Pollinations were also made by first self-pollinating and then, later cross-pollinating the same stigma . The reverse procedure was also followed,(i.e. first crossing then selfing). The time intervals between pollinations were 1,5 and 15 minutes. The corolla tube was removed before each pollination. Some of these flowers were left unpollinated to act as controls. Cross-pollinations and self-pollinations were replicated 32 times, using striga plants from both sources. Other types of pollinations were replicated 8 times. The pollinations were made between 17.00 and 18.00 h. Whole pistils were removed 16 hours later, fixed, macerated and stained in 0.1% aniline blue in phosphate buffer following the method of Kho and Baer (1968). The spread stylar tissue was examined through an incident fluorescence microscope using blue light for excitation. The pollen tubes fluoresced yellowish-green with their callose-plugs fluorescing more intensely.

Pistils were scored for the presence of normal pollen tubes inside the stylar tissue. Where tubes were running the whole length of the style, with callose-plugs at regular intervals, a compatible pollination was scored. If the pollen germinated but the pollen-tubes were short, deformed and with callose-plugs

dense and closely spaced, an incompatible pollination was scored. The percentage of pollen germination was determined for 5 compatible and 5 incompatible pollinations. The nuclei of pollen grains were observed, some after staining in acetocarmine, others after staining in a fluorescent dye specific to DNA called 4',6-diamidino-2-phenylindole (DAPI) (Hull, Hoshaw and Wang, 1982).

Plants from all the seed samples mentioned in Table 2.1 were self- and cross-pollinated separately. Inter-crosses were made between all the samples to test if any reproductive isolation has been operating to stop interbreeding between the different populations.

Observations were also made of natural pollination agents in Sudan in September and October 1982 in order to confirm that the same phenomena which were observed in the glasshouse cultivation in Britain also occured in the plants' natural environment. Some inflorescences were bagged in the field to prevent insects visiting the upper flowers; the lower flowers were left exposed as a control.

RESULTS

Morphology of the Flowers of *Striga hermonthica*:

The flowers of *S. hermonthica* are the largest and most showy of the agronomically important species. They are arranged spirally in conspicuous terminal spikes usually between 15-45 cm long. Their corolla tubes are erect at the base but bent outwards near the middle at an angle of almost 90°, so the widely-spreading purplish-red corolla lobes are arranged parallel to the axis in an obvious display. Longitudinal sections through the flower showed that the stigma is situated below the bend while the anthers are just above it. The inner surface of the middle

Fig. 3.1 Photograph of the middle part of a half flower of Striga hermonthica showing dense hairs on the inner surface of the corolla tube. The hairs prevent pollen from falling down the corolla tube. X 20

Fig. 3.2 Scanning electron micrograph of the dorsiventrally-flattened stigma of Striga hermonthica seen from the side. The receptive dorsal surface (on the left) is papillate while the ventral one is not. X 250

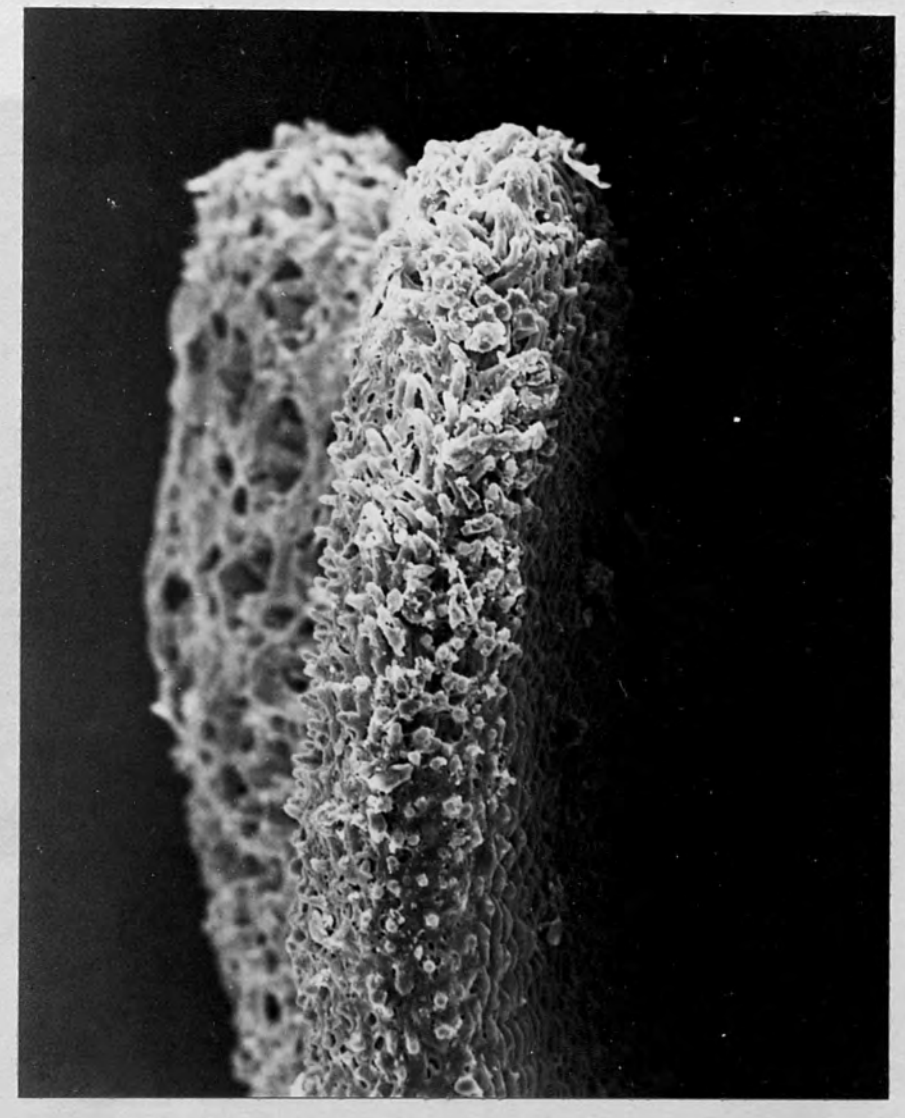


Fig. 3.3 A photograph showing the two sides of the stigma. On the left is the dorsal receptive side. On the right is the smoot, non-receptive side. X 20

section of the corolla tube is covered by stiff, upward-pointing hairs from above the bend to halfway down towards the base (Fig. 3.1) These hairs act as a barrier between the anthers and the stigma, and presumably exclude small Arthropods from the sweet nectar which is produced in abundance at the base of the corolla. The four stamens are epipetalous and dorsal, with very short filaments and comparatively large anthers. The anthers shed their masses of pollen which settle on the ventral side of the upper half of the corolla tube. The dense lining of hairs traps the pollen and stops it falling into the lower half of the corolla tube and onto the stigma. The ovary is surmounted by a short cylindrical style which terminates in an elongated, dark-green and tongue-like dorsiventrally flattened stigma. The dorsal side of the stigma (adaxial) is papillate and sticky while the ventral side is smooth (Figs. 3.2 and 3.3). This dorsal side almost adheres to the hairy inner wall of the corolla tube.

Effect of Pollen Source on Siphon Development:

All the flowers pollinated with their own pollen and also those pollinated using pollen from different flowers on the same spike failed to develop normal pollen-tubes. The pollen grains developed siphons but these were short and of the incompatible type, with closely spaced and prominant callose-plugs (Fig. 3.4) and some with swollen tips (Fig. 3.5). Flowers which were pollinated with pollen from flowers on spikes growing around host plants in other pots showed long pollen-tubes having regularly spaced callose-plugs which were easily detected in the stylar tissue using fluorescence microscopy (Fig. 3.6 and 3.7). The same results were obtained when crosses were made between plants of a different strain growing on the other host species. The percentage of non-germinating pollen grains for compatible crosses was (5 %- 8 %); for incompatible pollinations, the percentage was (3 %- 9 %). These results indicate that S. hermonthica is outbred due to self-incompatibility.

Fig. 3.4 Fluorescence micrograph of a stigma of Striga hermonthica 16 hours after self-pollination. The few germinating pollen grains produce only deformed pollen tubes with dense callose at the tips which appear to be related to the cessation of growth. X 400

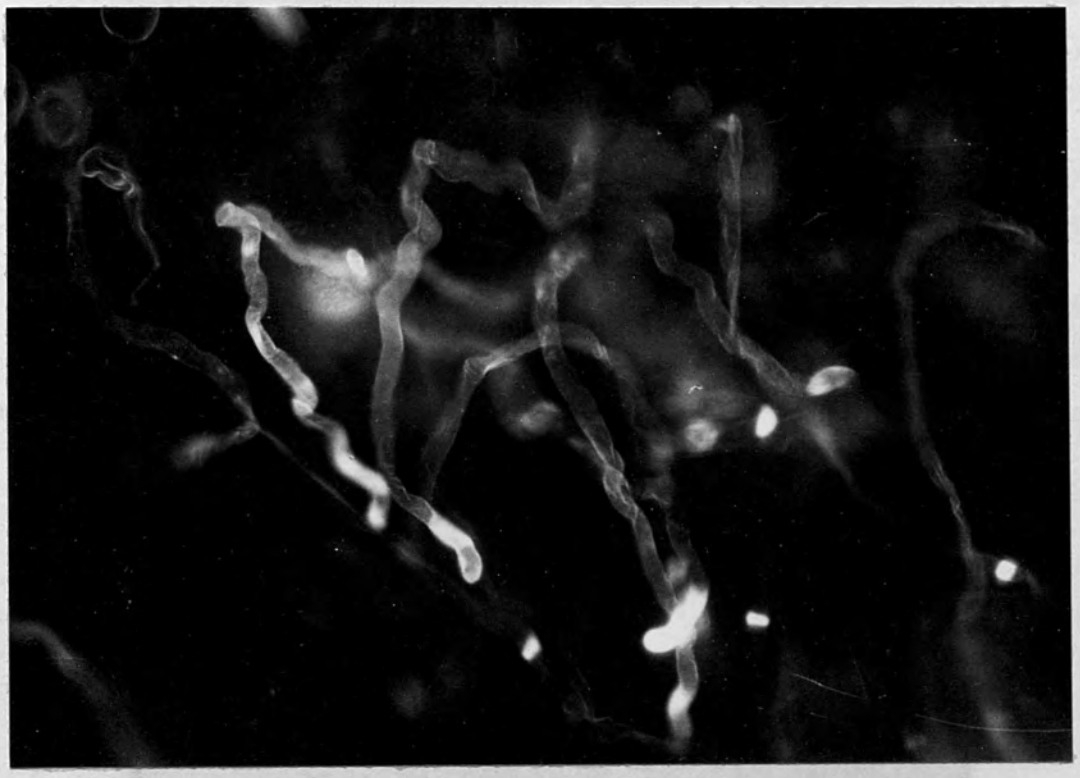


Fig. 3.5 Fluorescent micrograph the end part of pollen tubes after self-pollination. They show a swollen end just before bursting, indicating an incompatible reaction. X 290

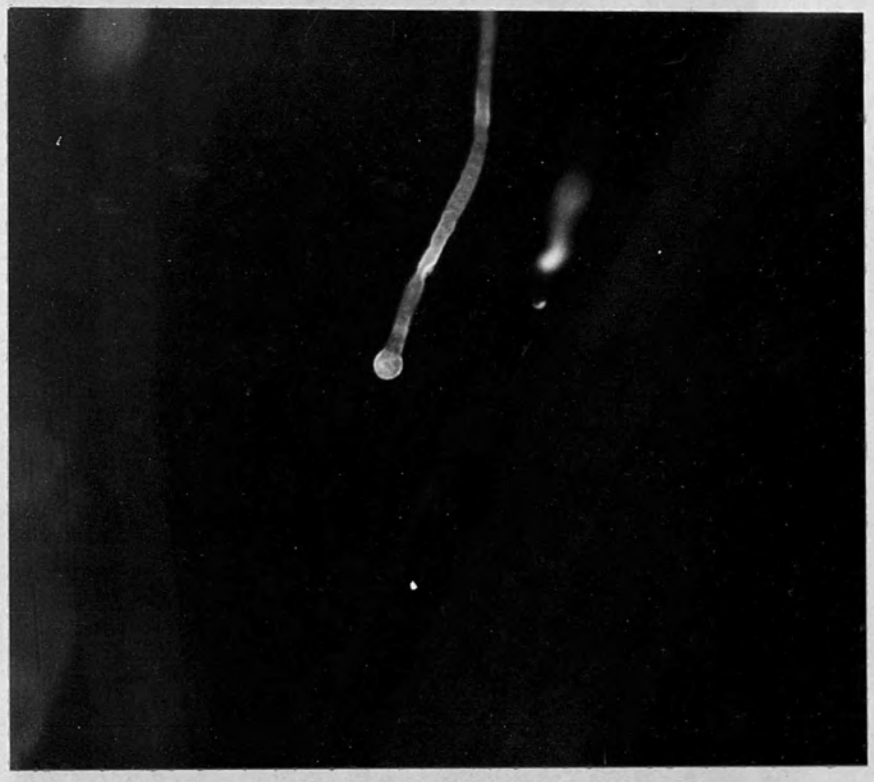


Fig. 3.6 Fluorescence micrograph of pollen tubes in a style of Striga hermonthica 16 hours after cross-pollination . The pollen tubes are straight and the callose plugs are widely spaced. X 290

Fig. 3.7 As Fig. 3.6 but showing pollen tubes further down the style.

Where several Striga shoots emerged around a single host plant it was found that their flowers were cross-compatible. This indicates that each shoot must be a separate plant which has developed from a different seed.

Receptivity of the Opposite Sides of the Stigma:

When compatible pollen was applied to the dorsal (upper) face of the flattened stigma, it developed normally, but when such pollen was carefully placed only on the ventral (lower) face it failed to develop. This indicates that pollination is only effective when compatible pollen reaches the papillate, sticky dorsal surface, for even compatible pollen placed on the ventral side failed to develop.

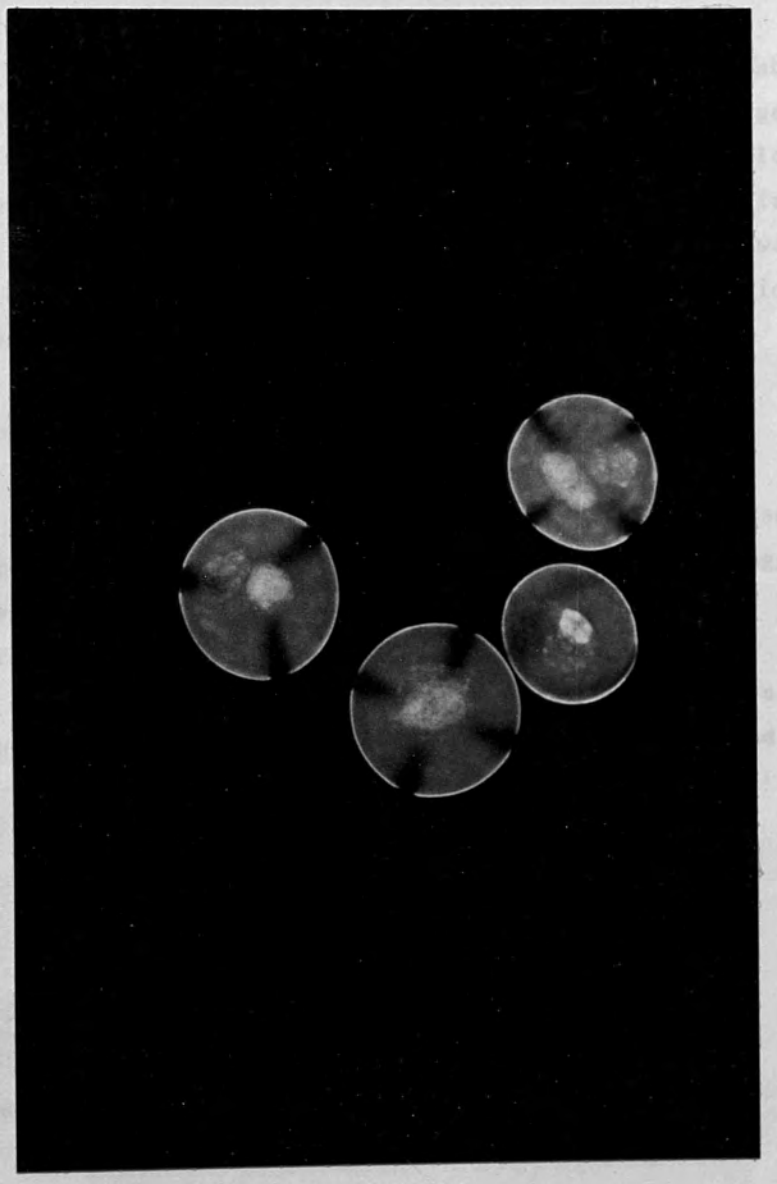
Effect of Pollination with a Mixture of Parental and Foreign Pollen:

When a mixture of self- and cross-pollen was used for pollination, some pollen grains managed to germinate and penetrate the stigma while others germinated but did not penetrate far into the style. This happened in all cases regardless of the sequence in which pollen was applied and of the time interval involved. Self-pollen placed on the stigma for up to 15 minutes before placing cross- pollen did not stop the stigma being receptive.

Nuclei in Pollen Grains:

At the time of release, the acetocarmine stain showed that each pollen grain contained a single ellipsoidal nucleus. However earlier stages, prior to the development of a fully sculptured exine, showed two nuclei. One was ellipsoidal: this is the

Fig. 3.8 Fluorescent micrograph of four pollen grains showing the presence of two nuclei in each; the generative nucleus fluoresces more intensely than the vegetative one, which is irregular in shape. It also shows that the pollen grains of S. hermonthica are dimorphic and are a mixture of tri- and tetracolpate types. X 600



generative nucleus which persists. The other was spherical: this is the vegetative nucleus which progressively degenerates during the maturation of the microspore until, by the time of anther dehiscence, only traces of it remained. When the fluorescent stain DAPI was used the vegetative as well as the generative nuclei were observed with the latter being less fluorescent and less regular in outline (Fig. 3.8).

Interfertility of the Different Populations:

All the plants studied from seed samples mentioned in Table 2.1 were found to be self-incompatible: self-pollinations gave little or no pollen-tube growth and the fruits failed to develop. On the other hand crosses, whether made between plants from different populations of the same host preference or between different host strains, were compatible i.e. all the populations are interfertile.

Observations on the Pollination of S. hermonthica in Sudan:

The flower spikes bagged to exclude visits by insect pollinators all failed to set seed, but many flowers on unbagged control spikes developed seed. This indicates that visits by insects are necessary for seed development.

The following butterflies were observed to visit flowers of Striga hermonthica in daylight hours in central Sudan:

Pieridae:
Catopsilia florella Fabricus
Pinacopteryx eriphia Godart
Belenois aurota Fabricus
Colotis protomedia Klug
C. halimede Klug

Nymphalidae
Junonia orithya L.
Cynthia cardvi L.

The behaviour of pollen deposited by visiting insects in nature was also observed. After newly-opened flowers had been visited by butterflies, they were later removed and their stigmas observed by fluorescence microscopy. It was found that many of the tubes grown from deposited pollen grains failed to reach the ovary: the proportion of pollen succeeding varied from very few to virtually all. The lower proportions of successful pollen development is presumed to be due to most of the pollen originating off the same plant, and therefore being incompatible. This can easily be explained by the way a butterfly visits an inflorescence. It takes a spiral route up a spike visiting one open flower after another, gathering pollen on its mouth parts as it does so. That also explains why not all the flowers produce seed in a striga plant even though most, if not all of them will be visited by potential pollinators. It was found that only 60% of the capsules produced seed in the fields around Wad Medani; a smaller percentage was noted elsewhere.

DISCUSSION

The showy nature of the flowers of Striga hermonthica indicates that the species is primarily adapted for animal pollination. The floral syndrome suggests that only long-tongued insects will be effective pollinators since the long, bent and narrow corolla tube prevents short-tongued insects reaching the nectar. The seven species of diurnal Lepidoptera which were seen to visit S. hermonthica flowers satisfied the specifications for being effective pollinators. Within the flower the spatial separation of the anthers and the stigma, the hiding of the receptive side of the latter, the bend in the corolla tube and the abundance of hairs inside the tube near the bend together act to prevent automatic self-pollination. All these factors, along with self-incompatibility we have demonstrated, act to promote outbreeding.

Self-incompatibility in S. hermonthica is effected during the growth of the siphon down the stigmatoid tissue of the style. It thus corresponds to the "gametophytic system" (Lewis, 1979) which is the type already reported in other species of the family Scrophulariaceae. This conclusion is also supported by the "binucleate" pollen. Bi-nucleate pollen is known to be associated with gametophytic incompatibility systems in angiosperms (Brewbaker, 1957).

The interfertility of plants from widely separated populations, in the most extreme case (Gambia and Ethiopia) 6000 kilometers apart, indicates that there are likely to be no barriers to gene exchange throughout the Sahel and possibly throughout the the entire range of the species. Equally interesting is the demonstration that the plants from the single sample of the "millet strain" are interfertile with all the populations of the "sorghum strain".

The occurrence of outbreeding in Striga hermonthica provides an explanation for some of the observed variation within populations in gross morphology (Musselman et al., 1979) and seed-coat ornamentation (Chapter 2). It may also account for the development of a degree of race specificity in S. hermonthica in response to different host species (Wilson-Jones,1955; King and Zummo, 1977), and to different varieties of those species (Doggett, 1965; Ramaiah, 1981). The development of various degrees of host-preference in local populations of the species throughout its range may be directly attributed to the action of natural selection upon variability for the genetic factors determining the physiology of the parasite, variability which has been preserved in populations by outbreeding.

This propensity for developing new strains of the parasite, able to preferentially attack particular host species or

varieties , casts doubt upon the effectiveness of the testing techniques widely used for screening cereals for striga resistance. These methods employ bulk samples of Striga hermonthica seed which, one may confidently predict, are mixtures of a large number of genotypes. Thus it is possible that apparently susceptible cereal varieties are resistant to some, possibly to many, of the genotypes of S. hermonthica present in a bulk seed sample. Their resistance is not detected because the plant succumbs to other genotypes of the parasite present in the mixture. Even sick-plot trials, because of the wide range of varieties of cereal species studied, will tend to favour the build-up of genetic diversity in the plot which, while not invalidating the empirical value of the trial, will obscure any genes present in the cereal which will afford protection against particular striga genotypes. The isolation and characterization of host-specific genotypes in Striga hermonthica should make it possible for the plant breeder to recognize genes in his cereal which offers degrees of protection against them. The method should be analogous to the present techniques for identifying genes offering protection against specific strains of fungal pathogens, and should be an improvement upon the present empirical techniques. Such work may provide an answer to the important question of whether it will ever be possible to breed cereals acceptably resistant to Striga hermonthica.

In the next Chapter I shall discuss the significance of the outbreeding nature of S. hermonthica when interpreting the results obtained using the different screening methods for identifying host cultivars resistant to the parasite attack.

[Part of this Chapter has been published (Safa, Jones and Musselman, 1984). A reprint will be found at the end of the Thesis]

CHAPTER 4

HOST PREFERENCE

INTRODUCTION

The demonstration of considerable variability in populations of Striga hermonthica for obvious morphological characters and for microscopic features (Chapter 2) suggests that the plant is likely to be similarly variable in the less readily observable qualities which determine its success as a parasite. The occurence of a strong self-incompatibility system promoting and maintaining variability, combined with natural selection favouring success in attacking a given host, makes it almost inevitable that many genes will be present, some of which confer success in establishment for one host, others for different hosts. When striga seed samples are tested against host varieties some of this variability is revealed.

In this chapter three screening methods are described which were used to study the variation in host preference of striga populations and in the time of emergence. Comparisons are made between the results obtained when using the different methods, and the results obtained when repeating the same experiment for three seasons using seeds from the same sample.

MATERIALS AND METHODS

Striga seeds of both strains, the "sorghum strain" and the "millet strain", which were collected from different parts of Sudan and Upper Volta were used in the tests. Each strain was tested on both host to test their specificities.

The three methods which were used to test the sorghum and millet varieties for their ability to stimulate and support S. hermonthica establishment are as follows. The first is the **double pot technique** described by Parker, Hitchcock and Ramaiah (1977) which tests the production of striga seeds' germination stimulants by the host roots' exudate. The second method is the **pot technique** described by Parker and Reid (1979) and refined by Wilson and Parker (1984). This method supplements the first one by also testing the ability of the host varieties to support the parasite throughout its different stages of development. The third method is the **polythene bag** technique developed by Parker and Dixon (1983). This method gives a visual insight into what is happening at the host-parasite contact point. These three methods are described in Appendices 3,4 and 5.

The double-pot and the pot methods were used, using seeds from the same seed samples of both host and parasite, for three seasons to look for any change in relative response with time.

A field trip was made to Sudan in November 1982 to visit the areas heavily infested with striga. Sorghum fields in the east and millet fields in the west were examined and observations made on the performance of cultivars. The comments of perceptive farmers were also noted where they had a bearing on the host-parasite relationship.

RESULTS

Effect of Age on S. hermonthica Seed Germination and Establishment:

Table 4.1 reports the results of a series of double pot experiments spread over 20 months. It shows that the difference in striga response to the varieties Feterita, CK60B and Dibaikri, which were known to be susceptible to striga, is not significantly different in the three years. Serena and Swarna although susceptible showed a significant drop in the number of striga seeds they stimulated to germinate by the summer of 1981 and 1982. In contrast, the number of germinated seeds increased with the passage of time when tested with the root-exudate of the resistant varieties N-13, Framida and Gadam El Hamam. Tetron, which is fairly tolerant, showed a drop in the number of germinating seed then an increase.

All the varieties showed an increase in susceptibility with age in the pot experiments (Table 4.2). The resistant varieties N-13 and Framida showed no sign of infection in 1981 season but they were as susceptible as Feterita and CK60B by 1982.

Host-specificity:

Table 4.3 gives the results of a double-pot experiment to test the response of the sorghum and the millet strains of striga against a range of sorghum and millet cultivars. The root exudate from the sorghum cultivars generally stimulated a larger number of striga seeds of the sorghum strain than the root-exudates from the millet cultivars. The same was true for the millet exudates and the millet strain. This applied for the susceptible cultivars only. The resistant cultivars from both host-species (Framida, IS 8686 and the millet P 2950) seem to stimulate few seeds of both strains to germinate.

Table 4.1 % Germination of striga seed collected in 1980 in response to root exudate of 9 sorghum varieties when repeated over three seasons: January 1981, August 1981 and August 1982. (4 replicates).

Variety Tested	Jan.1981	Aug.1981	Aug.1982
FETERITA	88, 90, 72, 83	88, 96, 97, 98	69, 79, 79, 65
(mean)	(83.2)	(94.7)	(73.0)
CK60B	100, 100, 99, 97	95, 89, 90, 100	80, 88, 69, 95
(mean)	(99.0)	(93.5)	(83.0)
SERENA	73, 25, 94, 87	57, 40, 27, 50	37, 17, 22, 28
(mean)	(69.7)	(43.5)	(26.0)
GADAM ELHAMAM	56, 42, 15, 11	95, 79, 89, 94	87, 97, 99, 96
(mean)	(31.0)	(89.2)	(94.7)
SWARNA	91, 52, 94, 30	50, 37, 63, 69	49, 47, 33, 42
(mean)	(66.7)	(48.0)	(42.7)
DIBAIKRI	98, 94, 99, 99	67, 63, 74, 80	92, 83, 80, 77
(mean)	(97.5)	(73.2)	(83.0)
TETRON	78, 83, 85, 94	73, 40, 46, 56	100, 90, 99, 92
(mean)	(85.0)	(53.8)	(95.2)
N-13	36, 31, 59, 46	79, 81, 93, 78	58, 69, 71, 67
(mean)	(43.0)	(82.7)	(66.2)
FRAMIDA	6, 0, 5, 1	6, 16, 13, 6	76, 61, 54, 48
(mean)	(4.5)	(10.2)	(59.7)

Source	DF	SS	MS	F
Variety	8	45168.2	5646.0	12.26
Season	2	47.8	239.2	1.58
Variety X Season	16	31634.6	1977.2	13.02 **
Residual	81	12300.5	151.9	
Total	107	89581.6		

L.S.D. (0.01) = 23.0.

Table 4.2 Number of Striga flowering spikes in pot experiments when tested against 4 sorghum varieties in two consecutive seasons. (5 replicates).

VARIETY	SUMMER 1981	SUMMER 1982
FETERETA	28, 21, 39, 29, 23	36, 28, 31, 29, 26
CK60B	26, 15, 32, 12, 10	25, 18, 29, 34, 24
N-13	0, 0, 0, 0, 0	20, 19, 32, 19, 38
FRAMIDA	0, 0, 0, 0, 0	19, 25, 15, 18, 22

The important feature of Table 4.3, as far as this study is concerned, is the few seeds of striga from both strains which respond to the other host species.

Response of S. hermonthica to Host Cultivars Using Three Methods of Screening:

Table 4.4 shows the results of testing two resistant varieties and two susceptible ones of sorghum against one sorghum strain of striga collected from Wad Medani, Sudan in 1980, using the three described methods.

Table 4.3 % Germination of striga seeds of the sorghum strain and the millet strain in response to root exudate of 8 sorghum varieties and 12 millet varieties. (4 replicates).

VARIETY TESTED	SORG.STR	MILL. STR.
WATER(CONTROL)	0	0
SORGHUMS:		
TUB-22	73, 84, 84, 82	9, 5, 17, 11
SORGO	94, 92, 82, 94	25, 28, 14, 13
GADAM ELHAMAM	90, 85, 95, 85	13, 13, 13, 16
FRAMIDA	11, 18, 5, 5	0, 5, 22, 21
SWANRA	57, 53, 63, 49	3, 2, 4, 0
CK60B	84, 70, 87, 89	13, 9, 4, 10
N-13	79, 67, 58, 62	4, 8, 8, 2
IS-8686	4, 2, 4, 2	2, 2, 2, 2
MILLETS:		
ACC 29	2, 2, 10, 16	38, 47, 47, 52
EX-BORNU	5, 2, 11, 13	63, 58, 69, 75
P-2950	0, 0, 0, 0	7, 15, 3, 3
ACC 30	6, 5, 9, 5	25, 34, 58, 42
ACC 37	5, 2, 4, 1	39, 44, 45, 41
ACC 64	0, 0, 3, 6	66, 43, 51, 47
ACC 182	0, 0, 0, 5	34, 41, 50, 44
ACC 58	4, 8, 5, 6	25, 25, 20, 14
ACC 148	0, 0, 0, 0	11, 19, 21, 12
ACC 42	0, 0, 0, 0	13, 10, 10, 6
ACC 39	0, 0, 0, 0	27, 41, 16, 30
ACC 40	0, 0, 0, 4	22, 26, 37, 27

Table 4.4 Comparison of the results when testing one strain of striga on 4 cultivars of sorghum using double pot, pot and polyethene bag techniques. (4 replicates)

Method Variety	Duoble pot (% germination)	Pot (number of striga shoots)	Polyethene bag (number of established striga shoots)
N-13	44	0	10
CK60B	99	17	12
FRAMIDA	10	0	1
FETERETA	83	28	5

The main feature of Table 4.4 is that it shows that stimulating striga to germinate and supporting its establishment are two different processes. Framida and N-13, although they stimulated some young striga seeds, did not support its establishment in the pot experiments. The other interesting feature is the ability of striga to establish on the resistant variety N-13 in the polythene bags while it failed to do so in the pot experiment. The relative response of Framida was the same regardless of the method of screening.

Arranging the varieties in a descending order of susceptibility for each method of screening reveals the following:

Double-pot method: CK60B > Feterita > N-13 > Framida
Pot Method : Feterita > CK60B > Framida = N-13
Poly-Bag Method : CK60B > N-13 > Feterita> Framida

In the polyethene bags the "resistant" variety N-13 was more susceptible than Feterita which is a well known susceptible variety.

Time of Emergence Above Soil Level:

Table 4.5 shows the effect of growing five varieties of sorghum with each of two strains of striga in a pot experiment. The performance is determined by counting the number of striga shoots appearing above soil level in pots. Two "sorghum strains" of striga were used in this experiment: one from Shambat, Sudan and the other from Kambionse, Upper Volta, both collected in 1979. The first to appear was the Sudanese strain on the variety CK60B after 6 weeks from sowing. The Upper Volta strain appeared two weeks later on the same variety. On the sorghum variety N-13 the Sudanese strain appeared two weeks earlier than the Upper Volta one but two weeks later than that on CK60B. On Gadam ElHamam both strains appeared on the same week (the 8th). On Framida and IS 8686 it took the Sudanese strain 10 weeks to appear while the Upper Volta one took 12 weeks. In all cases only some of the striga shoots that emerged managed to survive and reach the flowering stage. The maximum number was found on CK60B. Most of the striga seedlings died before they turned green while others died after surviving for up to 6 weeks. There are small but clear differences between the performance of the two striga strains even though both were adapted to sorghum.

Table 4.5. Relative performance of two striga strains in a pot experiment involving 5 sorghum cultivars

CULTIVAR	STRIGA ORIGIN	AVERAGE NUMBER OF STRIGA SHOOTS PER POT (4 REPLICATES)			
		10 AUG.	24 AUG.	11 SEPT.	27 OCT.*
N-13	SS	1.0	9.0	24.0	1.0
	US	0.0	2.0	6.75	0.0
IS 8686	SS	0.0	5.25	3.0	0.25
	US	0.0	0.75	0.75	0.0
CK60B	SS	12.75	55.5	30.25	4.75
	US	3.25	30.0	25.0	3.0
GADAM EL HAMAM	SS	1.0	4.0	8.5	0.25
	US	0.25	3.0	7.5	1.25
FRAMIDA	SS	0.25	3.5	7.25	1.25
	US	0.0	0.0	0.75	0.0

SS= Sudanese strain (from Shambat, Sudan)

US= Upper Volta strain (from Kambionse, Upper Volta)

(both strains were collected from sorghum fields in 1979.

Sowing date 15th June 1982.

* flowering by this time.

DISCUSSION

The increase in the susceptibility of the reputedly resistant varieties, such as Framida and N-13, as the striga seeds age (Table 4.1) agrees with the observations of Vallance (1950). He reported that older seeds of striga respond more readily to germination stimulants. The explanation he gave was that more seeds finish their after-ripening the longer they are kept, but in turn this indicates the existance of a number of genotypes, having different after-ripening requirements, in his seed samples. The increase in susceptibility of Framida and N-13 with the age of striga seed (Table 4.2) indicates that the genotypes specific to these varieties require longer after-ripening periods. The small number of seeds of the sorghum strain that can be stimulated to germinate by the millet root exudate, and vice versa, (Table 4.3) provides more evidence for the existance of a mixture of genotypes in individual populations. The development of host-specific strains (King and Zummo, 1977), seems to have been due to the act of natural selection. When the striga populations are subjected to one host species only for a long time, like the millet in the west of Sudan, this has favoured selection of genes governing effectiveness for attacking millet; while those whose genes were more adapted to sorghum or any other crop were gradually lost from the pool of long-lived seed in the soil. This has produced a striga population well adapted to a single host species which has appeared to be a single host strain, though it is likely to be far from uniform in view of its self-incompatibility (Safa, Jones and Musselman, 1984) and the reduced rate of natural selection associated with panmixis. The presence of a residue of genotypes, in each strain which are still able to attack an alternative host bears this out.

The same explanation can account for the appearance of the intraspecific physiological variants reported by Bebawi (1981). The difference in response of the different striga populations may be due to the fact that each striga population has had a different history of host plants and has been subjected to different environmental conditions. Both processes would result in genetic differences between the populations as reflected in the difference in time of emergence above soil level of the strains collected from Sudan and Upper Volta (Table 4.5), although they were grown on the same host varieties and under the same environmental conditions.

The presence of a mixture of genotypes in the striga populations and their maintainance through outbreeding gives a reasonable explanation for the recent breakdown of the immunity of the millet in the Gedarif area of Sudan. Millet was introduced to the area, unconciously, as a trap crop when the sorghum was heavily infested with striga. During the field trip to Gedarif the millet as well as the sorghum were found to be heavily infested in some places. The rare genes present in the striga population which confered the ability to attack millet have gradually multiplied. The virulant genes will then have spread through outbreeding and increased through the effect of natural selection. Alternatively, it is possible that seed of the millet strain of striga might have been introduced to the Gedarif area, and through hybridization, the adaptive genes have been diffused into the existing sorghum strain, since the two strains are fully interfertile (Chapter 3).

The difference in results obtained using different methods of screening (Table 4.4) reflects the complexity of the striga-host relationships. To understand the exact relationships one has to study the genetics of each step involved in the resistance reaction separately. This would give more insight into the characteristics the plant breeder has to breed for, rather than

the present tactic: an over-optimistic search for a "super-variety" containing all the genes responsible for all the mechanisms of resistance. Such a variety might not exist in reality.

[Some of the material presented in this Chapter was given by the author as a "discussion paper" to the 3rd International Symposium on Parasitic Weeds, Aleppo, Syria, in May 1984.]

CHAPTER 5

GENERAL DISCUSSION

The drastic reduction of the cereal crops' yield caused by the hemiparasite Striga hermonthica in tropical Africa led research workers to look for effective methods of control. Chemical means of control have received most attention because of the considerable resources of the agro-chemical companies and their heavy investment in research, but they have some serious disadvantages: apart from the high cost of the chemicals and the sophisticated equipment required for application, their effects were found to be variable under different environmental conditions. For these reasons this type of control is unlikely to be important or effective, at least as far as the small farmers are concerned.

Cultural control methods like trap- and catch-cropping, are costly and time-consuming. Catch-crops especially are impractical in areas where the growing season is too short to accomodate the catch-crop and then the main crop. Some of the trap-crops are not what the market wants. This makes the method less favoured since it is necessarily a long term solution because of the large pool of seeds in the soil and their longevity. In some areas, some trap-crops lost their immunity and became susceptible to striga attack, like the pennisetum millet in the east of Sudan.

Hand-weeding is not very effective since most of the damage occurs while the striga is still below ground. It is not beneficial to the immediate crop, but if carried out for a few seasons it can reduce the striga seed reserve in the soil. In heavily infested areas hand-weeding is not practical.

The biological control methods using herbivorous insect larvae and pathogens are still under test. It is too early to

make a final assessment, but theoretically this method will never give a 100% control: it can only help reducing the level of infestation.

Recently efforts have been concentrated on developing cultivars of the main cereal crops resistant to striga attack. Various methods of screening have been developed in order to identify resistant cultivars. These include sick- plot trials, testing in pots, extraction of stimulants by the double-pot technique and testing for resistance to attack by the polythene bag technique. The results obtained by applying these different testing techniques have been far from consistant. It is quite likely that they are measuring different aspects of the complex host-parasite interaction. The observed inconsistency may be due to any or all of three factors: variation in the experimental conditions, in the host plant or in the parasite. The experimental conditions can be, and normally are, kept fairly constant e.g. soil type, temperature and humidity. The host plant is normally a variety or a cultivar of a more or less uniform genetic make-up, since that is a prime requirement before release. This leaves us with the parasite as the most important source of inconsistant results. Unlike the hosts, it is not a specified genotype. It is a wild plant and subject to natural selection. We would expect it to exhibit variation in response to climate and host-preference. The morphological variations in every population I studied can be taken on its own to provide evidence for the lack of genetic uniformity and the existence of a large number of genotypes in individual S. hermonthica populations.

Flor (1956), working on the genetics of the flax-rust system, concluded that "for each gene conditioning reaction in the host there is a specific gene conditioning pathogenicity in the parasite". This implies that for a screening program to be effective both the parasite as well as the host should be

genetically uniform. The uniformity of both is essential for recognising the genes responsible for resistance in the host and the genes responsible for virulance in the parasite.

To reach a stage where we can establish a gene-for-gene relationships between sorghum or millet cultivars and S. hermonthica genotypes, the isolation and characterisation of specific striga genotypes are essential. This would be easy if one is working with fungal or bacterial pathogens, where cultures from single spores can produce enough material of uniform genetic make-up in a short period of time, but for S. hermonthica to set seed the parent plants must be genetically dissimilar, at least for the genes controlling compatibility, since it is self-incompatible and an obligate outbreeder. Dealing with a pathogen which is diploid and outbreeding makes it difficult, if not impossible, to produce pathogen material of uniform genetic make-up for testing purposes.

The failure in finding millet varieties 100% resistant to striga is not necessarily due to a lack of resistance genes in the host. Some varieties might have some resistance genes which protect them from certain striga genotypes, but not from all the genotypes present in the mixed seed samples used in the screening tests (ref. Chapter 4).

The striga seed samples used in the screening tests are normally collected from areas where the farmers are not worried about the purity of the cereal cultivars they are growing. They grow impure varieties which are mixtures of genotypes of the crop, in the same field or in neighbouring fields. In some areas both sorghum and millet are grown in the same field. Through cross-pollination accomplished by insects the genotypes of striga of different specificities will interbreed producing striga races virulant to a wide range of host genotypes. Collecting striga seeds from such fields to be used in the screening for

identifying resistant cultivars will not provide very useful information, because the seed sample may contain a number of striga genotypes each having a different host-preference.

To recognise resistance genes in the host varietes it is essential to obtain specific races of striga with uniform genetic make-up. Difficult as it may sound, due to the outbreeding habit of S. hermonthica, the power of natural selection should not be ignored. If the parasite is subjected to selection pressure by growing it with individual host varieties in isolation for a few seasons, each specified host cultivar will act as a genetic sieve to more or less isolate a specific race of striga. These specified races of striga should then be used as the starting material for identifying the different genes responsible for resistance in the different host cultivars and then transfer these genes into cultivars of desirable agronomic background.

Evidence for the feasibility of the above suggestion could be obtained from the results of Wilson-Jones (1955) which have been confirmed by King and Zummo (1977), Parker and Reid (1979) and in Chapter 4 in this thesis. The existance of strains of S. hermonthica specific to sorghum and to millet shows that these strains have been produced by subjecting the existing striga populations to one host species for a long time. The existance of intraspecific physiological strains reported by Bebawi (1981) and in Chapter 4 could also be due to the effect of selection on the local striga populations favouring races adapted to the local environmental conditions and to the host plant varieties grown in each locality.

Although it will take relatively a long time to work-out the genetics of the striga-host relationships, and longer still to breed new varieties using that knowledge, it should still be possible to manipulate the wild populations of S. hermonthica in infested areas to the advantage of the farmer. This could be done

in a similar way to the suggested development of the host-specific races of striga but using the whole crop rather than doing small-scale experiments. If we grow only one variety of the crop in the same area repeatedly, after a few seasons, we will have encouraged development of the race or races specific to that variety. When the variety begins to show severe losses we should replace it by another, tested for possessing different genes for resistance to the encouraged race or races of striga. This will ensure a low level of infestation, till the races specific to the new variety multiply and spread. This replacement strategy can be done repeatedly as long as the replacement variety is known to perform well against the currently prevalent striga genotype.

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APPENDIX 1

Observations upon 10 morphological characters in a population of Striga hermonthica growing in a test-plot at Wad Medani, Sudan in 1982

Key to abbreviations used:

pn : plant number
ll : leaf length (mm)
lw : leaf width (mm)
cl : corolla length (mm)
cw : corolla width (mm)
cc : corolla colour, 1= Pale pink, 2= medium pink, 3=dark pink
cm : corolla margin, 1= entire, 2= dentate
ccu: corolla colour uniformity, 1= uniform, 2= varigated
sl : sepal length (mm)
ssp: position of stigma relative to sepals, 1= above, 2= at or below calyx level
iil: inflorescence internode length (mm) [average of the lower most six internodes]

pn	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil
1	79	12	37	25	1	2	2	13	1	10
2	72	7	35	28	2	2	2	11	2	11
3	73	14	37	25	3	2	1	13	2	11
4	101	18	40	29	2	2	2	14	2	9
5	89	13	37	27	3	1	2	15	2	13
6	69	11	35	28	1	1	2	12	2	14
7	69	12	35	25	2	2	2	11	1	16
8	88	9	36	28	2	2	1	13	1	18
9	98	9	34	25	3	1	1	18	2	11
10	53	9	33	25	1	2	1	11	2	13
11	64	7	36	30	2	1	1	10	1	13

pn	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil
12	115	17	36	27	3	2	1	18	2	16
13	60	16	34	25	2	2	2	12	2	11
14	106	17	35	22	2	1	1	12	2	10
15	55	7	39	32	2	2	1	11	1	9
16	82	13	35	32	3	2	2	8	2	11
17	80	12	32	21	2	2	1	12	2	6
18	67	12	30	20	2	2	1	12	2	7
19	55	10	29	19	3	2	1	12	2	9
20	67	10	24	18	2	2	1	12	2	7
21	76	7	34	21	3	1	2	13	1	15
22	98	13	40	32	2	2	2	15	1	13
23	107	9	36	23	2	1	1	15	1	12
24	68	10	31	22	2	2	1	11	2	5
25	51	5	33	23	2	1	2	12	1	9
26	56	7	32	27	2	1	1	10	2	12
27	65	8	33	20	2	1	2	10	1	13
28	72	13	34	23	2	2	1	14	1	22
29	99	19	30	23	2	1	2	18	2	10
30	86	13	33	21	2	2	2	12	2	9
31	51	7	38	27	2	1	2	13	1	13
32	67	8	34	24	2	2	1	14	2	11
33	58	12	35	24	1	1	1	11	1	32
34	47	4	34	28	2	1	2	12	1	11
35	91	10	35	31	1	1	1	15	2	13
36	91	12	35	28	2	2	1	15	2	12
37	80	16	40	30	2	1	2	16	1	15
38	83	16	35	30	1	2	1	11	2	11
39	64	10	33	25	2	2	1	12	1	10
40	85	12	40	30	2	2	1	11	1	11
41	68	11	33	28	3	1	1	12	1	12
42	97	12	37	30	3	1	1	10	1	14
43	130	20	37	29	2	1	2	16	2	21
44	100	13	35	28	2	1	2	12	1	12
45	68	8	37	34	1	2	1	13	1	16

pn	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil
46	80	11	35	26	1	1	2	11	1	19
47	63	11	38	32	3	2	1	10	1	14
48	86	12	33	25	3	1	1	15	2	11
49	76	10	40	34	1	2	1	13	2	9
50	66	12	30	28	1	1	1	11	1	14
51	87	17	32	29	1	2	1	12	2	7
52	75	8	27	17	1	1	1	9	1	6
53	69	10	16	24	1	2	1	14	2	8
54	69	10	24	21	1	2	1	12	2	7
55	63	9	23	20	2	2	1	12	2	9
56	56	7	25	17	1	2	1	7	2	5
57	29	8	23	18	2	2	2	12	2	11
58	44	11	26	18	2	2	1	11	2	10
59	51	9	30	22	2	2	1	10	2	10
60	25	6	28	14	2	2	2	10	2	6
61	49	7	25	9	2	2	2	12	2	7
62	64	10	27	15	2	2	1	8	2	10
63	53	8	21	19	1	2	2	14	2	8
64	62	11	17	13	2	2	1	9	2	10
65	68	12	34	23	1	2	1	11	2	10
66	60	12	30	19	1	1	1	13	2	8
67	83	11	32	23	3	2	1	13	2	6
68	98	11	22	14	1	2	1	15	2	8
69	41	7	24	8	3	2	1	9	2	6
70	78	9	19	25	3	1	1	11	2	9
71	81	12	20	17	3	2	1	13	2	7
72	74	11	26	29	1	2	2	9	2	7
73	95	13	34	26	3	1	1	14	2	8
74	56	12	38	35	2	2	2	12	2	4
75	71	14	32	22	3	2	2	11	2	7
76	71	16	35	27	2	2	2	13	2	6
77	85	13	34	26	2	2	2	13	2	7
78	95	14	38	28	2	2	2	16	2	15
79	105	11	36	27	2	1	2	13	2	13
80	111	13	38	25	1	2	1	14	2	6

pn	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil
81	62	9	34	27	1	1	2	14	2	8
82	70	11	25	20	3	1	2	14	2	12
83	58	8	35	25	3	1	2	12	2	6
84	58	10	33	23	2	1	2	14	2	7
85	58	10	35	35	2	2	1	15	2	12
86	62	9	35	20	2	2	2	13	1	10
87	59	9	35	25	3	2	1	13	1	10
88	72	8	33	20	2	1	2	12	2	12
89	89	15	27	21	2	1	2	12	1	11
90	43	15	28	20	2	1	2	11	1	10
91	120	15	39	31	2	2	2	25	1	12
92	73	6	35	25	2	2	1	14	1	11
93	105	16	42	35	2	1	1	16	2	16
94	87	13	32	23	2	2	2	10	1	14
95	80	9	35	23	2	1	1	14	2	13
96	71	14	35	34	2	1	1	13	1	14
97	78	10	32	24	1	2	1	12	1	10
98	67	12	32	32	2	2	1	12	1	10
99	65	11	34	26	1	2	1	12	1	10
100	58	10	40	35	2	2	2	12	1	10
101	93	14	40	30	3	1	1	16	1	18
102	88	12	35	21	1	2	1	15	1	15
103	75	9	31	23	2	1	1	12	1	8
104	58	12	38	30	2	1	1	14	1	10
105	64	9	25	21	1	2	1	11	2	6
106	70	15	25	21	2	1	1	12	2	8
107	57	12	26	24	2	2	1	9	2	6
108	46	8	32	18	1	2	1	9	2	8
109	85	13	33	30	2	1	1	12	1	10
110	82	13	28	23	3	1	1	11	1	14

pn	ll	lw	cl	cw	cc	cm	ccu	sl	ssp	iil
111	88	12	41	36	2	2	2	16	2	12
112	82	8	37	31	3	1	1	12	1	14
113	80	15	27	27	2	2	2	12	1	16
114	89	11	30	25	2	2	2	12	1	16
115	76	14	34	27	3	2	1	12	1	10
116	92	15	30	23	2	2	2	12	1	10
117	66	16	30	26	1	1	1	10	2	13
118	72	9	40	32	2	2	1	15	1	11
119	70	10	36	25	1	2	1	11	2	13
120	65	10	46	30	2	2	1	13	1	11
mean	73.08	11.21	32.63	24.95				12.51		10.95
s.d.	18.78	3.05	5.48	5.42				2.37		3.95

APPENDIX 2

Measurements of 10 morphological features of Striga hermonthica plants grown in a glass-house at RHC, Egham, Surrey in 1983, from the seed samples detailed in Table 2.1.

ETHIOPIA

pn	11	lw	cl	cw	cc	cm	ccu	sl	pss	iil
1	84	11	29	18	3	1	2	12	2	7
2	77	12	17	16	3	2	2	10	1	16
3	80	12	31	15	2	2	2	14	2	13
4	82	10	26	14	2	1	1	9	1	6
5	88	10	25	25	3	2	1	12	1	8
6	81	11	25	18	2	2	2	11	1	4
7	73	13	26	17	1	1	2	13	2	8
8	76	12	24	26	1	2	1	15	1	14
9	81	8	21	12	3	1	1	14	2	10
10	73	9	19	11	1	1	1	8	2	11
11	60	10	15	14	2	2	1	12	2	16
12	82	11	31	15	3	1	2	10	1	15

SUDAN

1	99	15	29	25	1	2	1	14	1	8
2	73	15	34	33	3	2	2	10	2	7
3	84	16	36	31	2	2	2	13	1	8
4	53	15	28	27	3	1	1	12	1	6
5	68	13	26	9	1	1	1	9	2	6
6	86	18	18	12	3	2	1	11	2	10
7	78	11	21	21	3	1	1	12	2	8
8	80	9	26	25	1	2	2	9	2	9
9	109	16	19	19	1	1	1	12	2	7
10	59	13	21	18	2	2	1	11	2	9
11	86	13	17	17	2	1	2	11	1	12
12	96	14	18	20	2	1	1	9	1	7

NIGERIA

pn	ll	lw	cl	cw	cc	cm	ccu	sl	pss	iil
1	92	6	25	17	1	1	1	14	2	8
2	101	6	26	18	2	1	2	12	2	12
3	67	7	28	18	2	1	1	15	2	6
4	67	7	31	20	1	2	1	16	2	8
5	91	6	26	21	3	1	2	9	1	12
6	87	6	26	26	2	2	2	12	2	11
7	79	6	19	18	3	2	1	14	1	10
8	111	9	17	18	1	2	2	9	1	12
9	102	10	17	25	2	1	1	10	1	13
10	93	7	25	26	3	2	1	6	1	14
11	93	11	23	18	2	1	2	11	2	15
12	89	7	25	18	2	1	1	12	2	18

NIGER

1	150	12	24	17	3	1	2	12	2	12
2	80	11	23	15	3	1	1	11	2	14
3	110	11	28	19	1	2	2	14	2	22
4	89	9	24	16	2	1	1	10	2	13
5	71	10	25	17	3	1	1	9	1	16
6	106	7	23	16	1	2	1	8	2	19
7	131	9	25	17	2	2	2	11	2	14
8	89	10	22	16	2	2	1	9	1	11
9	150	11	22	19	2	2	2	12	2	14
10	123	9	21	21	1	2	2	9	2	12
11	104	10	22	18	2	1	2	8	1	5
12	83	9	21	15	2	2	2	13	1	9

MALI

pn	ll	lw	cl	cw	cc	cm	ccu	sl	pss	iil
1	66	6	25	17	3	1	2	14	1	19
2	101	7	17	16	1	2	2	12	2	13
3	69	8	20	21	2	2	1	11	2	12
4	78	5	26	19	2	1	1	13	1	5
5	78	6	24	21	2	1	1	18	1	10
6	87	7	27	19	2	1	2	11	2	9
7	83	7	31	20	3	2	1	10	1	13
8	102	6	26	17	2	1	2	18	1	11
9	66	9	22	22	1	1	2	12	2	15
10	66	6	24	26	2	2	2	12	1	11
11	66	8	35	31	3	2	1	12	1	10
12	102	7	32	19	2	2	2	11	2	11

UPPER VOLTA

1	101	8	27	22	3	1	1	14	9	32
2	86	8	31	24	1	2	2	12	9	11
3	62	7	32	23	2	2	1	7	1	13
4	78	8	22	23	2	1	2	10	2	14
5	87	9	27	21	2	1	2	12	2	11
6	64	9	19	19	2	2	1	15	1	10
7	58	6	23	25	3	1	1	9	1	9
8	80	5	21	18	2	1	2	9	2	11
9	96	8	21	21	3	1	1	13	1	6
10	94	9	28	18	2	2	2	16	1	7
11	89	6	20	21	2	2	2	12	1	9
12	99	8	26	19	1	2	1	14	1	6

GHANA

pn	ll	lw	cl	cw	cc	cm	ccu	sl	pss	iil
1	82	7	21	17	2	1	1	9	1	12
2	93	6	27	18	1	2	1	9	1	14
3	73	6	31	15	2	1	2	12	2	21
4	69	6	18	17	2	1	1	8	2	12
5	98	7	26	21	2	2	1	12	2	11
6	82	8	31	22	3	1	2	9	2	10
7	78	9	21	16	1	2	2	8	2	16
8	54	8	24	19	2	1	2	11	1	16
9	92	8	20	17	2	1	2	12	1	9
10	101	6	19	18	2	1	1	9	1	14
11	81	8	17	16	2	1	1	11	2	8
12	73	6	12	19	2	1	1	12	2	7

GHAMBIA

1	78	6	32	23	1	1	1	12	1	6
2	89	8	21	19	1	2	1	11	1	10
3	79	8	18	16	1	1	2	13	1	8
4	80	6	24	26	2	1	1	14	2	7
5	78	6	17	14	2	2	1	15	2	7
6	90	7	17	17	2	2	2	11	2	10
7	72	6	24	23	1	1	2	12	1	8
8	49	7	15	16	1	1	2	13	2	11
9	88	7	25	23	3	2	1	9	2	8
10	89	8	22	17	3	1	1	11	1	8
11	95	8	26	21	3	2	2	13	1	6
12	69	10	17	16	1	1	1	9	2	8

APPENDIX 3

THE DOUBLE-POT TECHNIQUE

(modified from Parker, Hitchcock and Ramaiah, 1977)

Seeds of the crops to be tested (sorghum or millet) are surface sterilized in a 1% sodium hypochlorite solution for about 20 minutes. They are then washed thoroughly in distilled sterile water and placed to germinate on wet 9cm glass fiber (g/f) filter paper in a sterile plastic petri-dish. 10 pregerminated seeds are sown in acid washed sand in 2.5" diameter plastic flower-pots with perforated bottoms. The bottom of each pot is covered with a glass-fiber filter paper disc to stop sand from pouring through the holes. Each pot is then placed in a cup of a similar size to collect the solution draining through the holes of the pot.

Pots are watered daily with about 15 ml of distilled water or when necessary. The pots are kept in a green house at a temperature of about 30° C, and provided with supplementary light using 400W mercury lamps. Seedlings are left to grow till their roots penetrated the perforations of the flower pot and are emmersed in the solution in the cup (Fig. App. 1). This solution is then used to test the cultivars ability to stimulate striga seeds' germination.

S. hermonthica seeds are surface sterilized in a 1% sodium hypochlorite solution for about 5 minutes, and then washed thoroughly with distilled water using a Buchner funnel lined with filter paper (because of the small size of the seeds). About 25 surface sterilized seeds are placed on each of 8mm g/f filter paper discs. Discs are then placed on saturated g/f filter paper in plastic petri-dishes. The dishes are then put in polythene bags and are kept in a dark incubator at a temperature of about 23° C for 10-15 days.

Four discs of the pretreated striga seed are then transfered to each of four petri-dishes containing filter papers saturated with the root exudate of the variety to be tested. 48 hours later the number of germinating seed on each dics are counted. Percentage germination is calculated as a percentage of the total number of seeds.

Fig. App. 1 Shows the assembly of the double-pot technique and the stage at which I collected the host root exudate for the screening tests, where the roots of the sorghum or the millet penetrated the holes of the upper pot and became imersed in the solution in the cup below.

APPENDIX 4

POT TECHNIQUE

(Modified from Parker and Reid, 1979)

Sterilized John Innes No.1 compost is used without adding any extra fertilizer to keep the nitrogen level to the minimum. 7" inches diameter plastic pots are filled with soil to about 2" from the top. 100-200 striga seeds sprinkled on the top are thoroughly mixed with top part of the soil in each pot using a spatula. Five seeds of each variety of sorghum or millet are placed on the surface and are then covered with about an inch of the same soil type. After emergence the seedlings are then thinned to one plant per pot. (Fig. App. 2).

Pots are kept standing in trays on the floor of a green house which is kept at a temperature above 25°C all the time. Supplimentary light is provided for 12 hours daily using 400W mercury lamps suspended 1.7 m above floor level. Watering is carried out when necessary by flooding the trays in which the pots are standing.

Fig. App.2 Shows the standard pot-technique used for screening for the millet's ability to prevent striga establishment.

APPENDIX 5

The Polythene Bag Technique

Glass fiber filter paper sheets (5"X10") are inserted inside 6"X11" polyethene bags under sterile conditions and wetted with 15 ml of sterile distilled water using a disposable syringe. Surface-sterilized dry striga seed (cf. Appendix 3) are scattered on one side of the wetted sheet and are spread evenly by brushing the surface of the bag using a medium-soft painting brush. Bags are then suspended in square buckets after rolling the top part around a bamboo rod which is just longer than the width of the bucket. The striga seeds are kept for 10-15 days for pretreatment. The bottom corners of each bag are cut to drain the solution in which the striga seed are pretreated. A pregerminated, surface-sterile (cf. Appendix 3) host seed is inserted inside each bag, about an inch from the top through a longitudinal slit made on the side where the striga seeds are placed. Bags are extra supported by two bamboo rods stuck to the back of each bag to keep them spaced and upright (Fig. App. 3).

Bags are then suspended in the square bucket after filling it with a nutrient solution (Parker and Dixon, 1983) to about two inches from the bottom, enough to cover the cut corners of the bags. This is to allow the nutrient solution travel up the filter paper sheet and reach the growing host seed. The buckets are painted black and are provided with a black, folding lid to give the maximum exclusion of light (Fig. App. 4). They are then kept in a glass house at a temperature above 25 ^{o}C and provided with supplimentary light using 400 W mecury lamps for 12 hours daily.

Fig. App.3 The polythene bag technique assembly showing the S. hermonthica established on the roots of a sorghum plant.

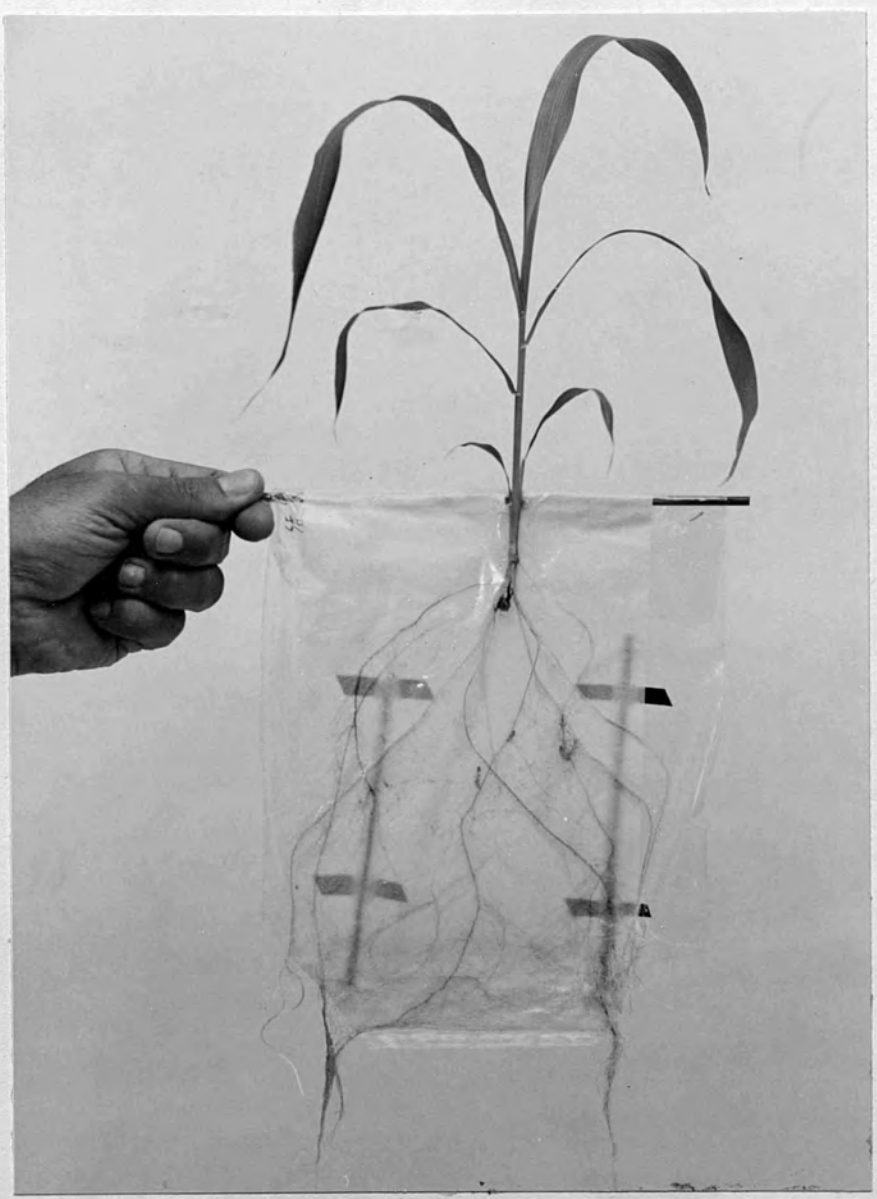

Fig. App.4 In the polythene bag technique the bags (Fig. App.3) are suspended in a nutrient solution in a darkened bucket. The bucket is provided with a folding black lid to stop light from reaching the roots.

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MECHANISMS FAVOURING OUTBREEDING IN *STRIGA HERMONTHICA* [SCROPHULARIACEAE]

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SUMMARY

Self-incompatibility has been demonstrated in two samples of the parasitic flowering plant, *Striga hermonthica*, originating from Upper Volta and Sudan. Compatibility was determined by fluorescence microscopy of stigmatic preparations following controlled pollinations. Self incompatibility was associated with floral adaptations favouring pollination by long-tongued insects such as butterflies. Outbreeding accounts for the variability of *S. hermonthica*, both in morphology and in host-specificity. Genetic variation in host specificity imposes limits upon the effectiveness of the present methods of testing tropical cereals for resistance to *S. hermonthica*.

Key words: *Striga hermonthica*, parasitic weed, outbreeding, self-incompatibility, host-specificity.

INTRODUCTION

Striga hermonthica (Del.) Benth. is widespread in Africa attacking *Sorghum bicolor* Moench. (sorghum) *Pennisetum americanum* Rich. (pennisetum millet) and many other crops. Because of the high cost and relative ineffectiveness of cultural and chemical control measures (Russell, 1978) efforts have been directed towards breeding for resistant cultivars. Yet, not a single variety of sorghum, pennisetum or any other cereal has been found to have 100% resistance to *S. hermonthica*. Some cultivars show acceptable field resistance in certain areas but are susceptible elsewhere (Wilson-Jones, 1955; Ramaiah, 1981). Even in one and the same area, the performance of some cultivars varies from one season to another (Doggett, 1965). Wilson-Jones (1955) reported that *S. hermonthica* has at least two strains in Sudan: one attacking sorghum and the other attacking pennisetum. This variation in host-preference was confirmed by Parker & Reid (1979) and Ramaiah (1981). A wide range of variation in plant size and corolla structure and colour within Nigerian populations of *S. hermonthica* was reported by Musselman,

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Nickrent, Mansfield & Ogborn (1979). Seed coat ornamentation is also variable within populations (Jones & Safa, 1982). This wide range of variation in plant form and behaviour implies that the species is outbreeding as suggested by Parker (reported in Musselman *et al.*, 1979).

Musselman, Parker & Dixon (1982) self-pollinated and intercrossed some *S. hermonthica* plants grown from seed of a strain specific to sorghum and noticed that only the crossed flowers set seeds. The breeding system, because it determines the variation of this species, has an important bearing upon the plant-breeder's ability to select cereal lines reliably resistant to the parasite. We report here our investigations of the floral biology and breeding system of *S. hermonthica* and discuss their significance for breeding cereals resistant to *Striga*.

MATERIALS AND METHODS

Striga hermonthica plants were raised in glasshouse at Egham, Surrey, UK, from seed collected in 1979 in Upper Volta, using the method of cultivation described by Parker & Reid (1979). Supplementary lighting was given to the host plant for 12 h daily by 400 W mercury vapour lamps suspended 2 m above the pots. Two strains were used: one collected from plants attacking sorghum and the other from plants attacking pennisetum millet. Sorghum cv. CK60B and pennisetum cv. Ex Bornu were used as hosts in our experiments; single plants were grown in pots containing the appropriate strain of *Striga*. During the period when the observations were made the night temperature in the glasshouse was maintained at about 25 °C. When flowering commenced the glasshouse was kept closed to reduce the possibility of stray visits by insects. Each flower spike emerging in a pot was treated as a separate entity for experimental purposes. Self- and cross-pollinations were made by dusting the stimatic surfaces with parental or with foreign pollen using a brush and by carefully placing parental or foreign pollen on either side of the dorsoventrally flattened stigma using a needle. Pollinations were also made by first self-pollinating and then, later, cross-pollinating the same stigma. The reverse procedure was also followed, (i.e. first crossing then selfing). The time intervals between pollinations were 1, 5 and 15 min. The corolla tube was removed before each pollination. Some of these flowers were left unpollinated to act as controls. Cross-pollinations and self-pollinations were replicated 32 times, using *Striga* plants from both sources. Other types of pollinations were replicated eight times. The pollinations were made between 17.00 and 18.00 h. Whole pistils were removed 16 h later, fixed, macerated and stained in 0·1 % aniline blue in phosphate buffer following the method of Kho & Baer (1968). The spread stylar tissue was examined through an incident fluorescence microscope using blue light for excitation. The pollen tubes fluoresced yellowish-green with their callose-plugs fluorescing more intensely.

Pistils were scored for the presence of normal pollen tubes inside the stylar tissue. Where tubes were running the whole length of the style, with callose-plugs at regular intervals, a compatible pollination was scored. If the pollen germinated but the pollen tubes were short, deformed and with callose-plugs dense and closely spaced, an incompatible pollination was scored. The percentage of pollen germination was determined for five compatible and five incompatible pollinations. The nuclei of pollen grains were observed after staining in acetocarmine.

Observations were also made of natural pollination agents in Sudan in September and October 1982, and to confirm that the same phenomena which were

observed in the glasshouse cultivation in Britain also occurred in the plants natural environment. Some inflorescences were bagged in the field to prevent insects visiting the upper flowers; the lower flowers were left exposed as controls.

Results

Morphology of the flowers of Striga hermonthica

The flowers of *S. hermonthica* are the largest and most showy of the agronomically important species of the genus. They are arranged spirally in conspicuous terminal spikes usually between 15 to 45 cm long. Their corolla tubes are erect at the base but bent outwards near the middle at an angle of almost 90°, so the widely-spread purplish-red corolla lobes are arranged parallel to the axis in an obvious display. Longitudinal sections through the flower showed that the stigma is situated below the bend while the anthers are just above it. The inner surface of the middle section of the corolla tube is covered by hairs from above the bend to halfway down towards the base. These hairs act as a barrier between the anthers and the stigma, and presumably exclude small arthropods from the sweet nectar which is produced in abundance at the base of the corolla. The four stamens are epipetalous and dorsal, with very short filaments and comparatively large anthers. The anthers shed their masses of pollen which settle on the ventral side of the upper half of the corolla tube. The dense lining of hairs traps the pollen and stops it falling into the lower half of the corolla tube and onto the stigma. The ovary is surmounted by a short cylindrical style which terminates in an elongated, dark-green and tongue-like dorsiventrally flattened stigma. The dorsal side of the stigma (adaxial) is papillate and sticky while the ventral side is smooth [Fig. 1(a), (b)]. This dorsal side almost adheres to the hairy inner wall of the corolla tube.

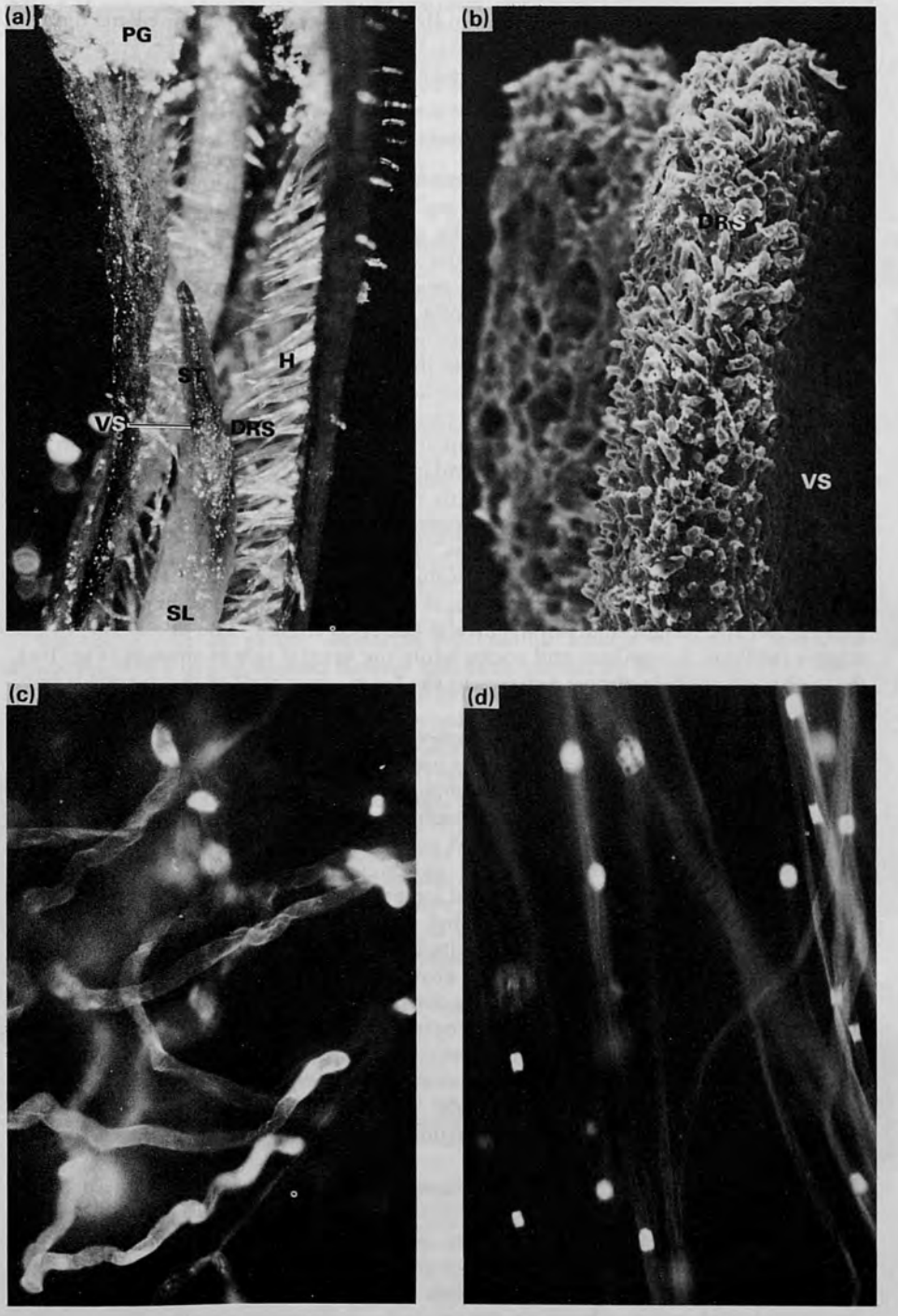
Effect of pollen source on siphon development

All the flowers pollinated with their own pollen and also those pollinated using pollen from different flowers on the same spike failed to develop normal pollen tubes. The pollen grains developed siphons but these were short and of the incompatible type, with closely spaced and prominant callose-plugs [Fig. 1(c)]. Flowers which were pollinated with pollen from flowers on spikes growing around host plants in other pots showed long pollen tubes having regularly spaced callose-plugs which were easily detected in the stylar tissue using fluorescence microscopy [Fig. 1(d)]. The same results were obtained when crosses were made between plants of a different strain growing on the other host species. The percentage of non-germinating pollen grains for compatible crosses was 5 to 8 and for incompatible pollinations 3 to 9. These results indicate that *S. hermonthica* is outbreeding due to self-incompatibility.

Where several *Striga* shoots emerged around a single host plant it was found that their flowers were cross-compatible. This indicates that each shoot must be a separate plant which has developed from a different seed.

Receptivity of the opposite sides of the stigma

When compatible pollen was applied to the dorsal (upper) face of the flattened stigma, it developed normally, but when such pollen was carefully placed only on the ventral (lower) face it failed to develop. This indicates that pollination is only effective when compatible pollen reaches the papillate, sticky dorsal surface, for even compatible pollen placed on the ventral side failed to develop.


(a)
PG
ST
H
VS
DRS
SL
(b)
DRS
VS
(c)
(d)

Effect of pollination with a mixture of parental and foreign pollen

When a mixture of self- and cross-pollen was used for pollination, some pollen grains managed to germinate and penetrate the stigma while others germinated but did not penetrate far into the style. This happened in all cases regardless of the sequence in which pollens were applied and of the time interval involved. Self-pollen placed on the stigma for up to 15 min before placing cross-pollen did not stop the stigma being receptive.

Nuclei in pollen grains

At the time of release, each pollen grain contains a single ellipsoidal nucleus; earlier stages, prior to the development of a fully sculptured exine, show two nuclei. One is ellipsoidal: this is the generative nucleus which persists. The other is spherical: this is the vegetative nucleus which progressively degenerates during the maturation of the microspore until, by the time of anther dehiscence, only traces of it remain.

Observations on the pollination of S. hermonthica *in the Sudan*

The flower spikes bagged to exclude visits by insect pollinators all failed to set seed, but many flowers on unbagged control spikes developed seed. This indicates that visits by insects are necessary for seed to be set.

The following butterflies were observed to visit flowers of *S. hermonthica* in daylight hours in central Sudan: Pieridae: *Catopsilia florella* Fabricus, *Pinacopteryx eriphia* Godart, *Belenois aurota* Fabricus, *Colotis protomedia* Klug and *C. halimede* Klug; Nymphalidae: *Junonia orithya* L., and *Cynthia cardvi* L.

The behaviour of pollen deposited by visiting insects in nature was also observed. After newly-opened flowers had been visited by butterflies, they were later removed and their stigmas observed by fluorescence microscopy. It was found that many of the tubes grown from deposited pollen grains failed to reach the ovary: The proportion of pollen succeeding varied from very few to virtually all. The lower proportions of successful pollen development is presumed to be due to most of the pollen originating off the same plant, and therefore being incompatible. This can easily be explained by the way a butterfly visits an inflorescence. It takes a spiral route up a spike visiting one open flower after another, gathering pollen on its mouth parts as it does so. That also explains why not all the flowers produce seed in a *Striga* plant even though most, if not all of them will be visited by potential pollinators. It was found that only 60 % of the capsules produced seed in the fields around Wad Medani; a smaller percentage was noted elsewhere.

Fig. 1. (a) Photograph of the middle part of a half flower of *Striga hermonthica* showing dense hairs on the inner surface of the corolla tube. The hairs prevent pollen from falling down the corolla tube. × 16. DRS, dorsal receptive surface; H, hairs; PG, pollen grains; SL, style; ST, stigma; VS, ventral surface. (b) Scanning electron micrograph of the dorsiventrally-flattened stigma of *S. hermonthica* seen from the side. The receptive dorsal surface is papillate while the ventral one is not. × 200. Abbreviations as in (a). (c) Fluorescence micrograph of a stigma of *S. hermonthica* 16 h after self-pollination. The few germinating pollen grains produce only deformed pollen tubes with dense callose at the tips which appear to be due to cessation of growth. × 320. (d) Fluorescence micrograph of pollen tubes in a style of *S. hermonthica* 16 h after cross-pollination. The pollen tubes are straight and the callose plugs are widely spaced. × 232.

DISCUSSION

The showy nature of the flowers of *S. hermonthica* indicates that the species is primarily adapted for animal pollination. The floral syndrome suggests that only long-tongued insects will be effective pollinators since the long, bent and narrow corolla tube prevents short-tongued insects reaching the nectar. The seven species of diurnal Lepidoptera which were seen to visit *S. hermonthica* flowers satisfied the specifications for being effective pollinators. Within the flower the spatial separation of the anthers and the stigma, the hiding of the receptive side of the latter, the bend in the corolla tube and the abundance of hairs inside the tube near the bend together act to prevent automatic self-pollination. All these factors, along with the self-incompatibility we have demonstrated, act to promote outbreeding.

Self-incompatibility in *S. hermonthica* is effected during the growth of the siphon down the stigmatoid tissue of the style. It thus corresponds to the 'gametophytic system' (Lewis, 1979) which is the type already reported in other genera of the family Scrophulariaceae. This conclusion is also supported by the 'binucleate' pollen type for, even though only one nucleus is actually present at maturity, the development of the pollen grain shows a two-nuclear stage before one aborts. Binucleate pollen is known to be associated with gametophytic incompatibility systems in angiosperms (Brewbaker, 1957).

The occurrence of outbreeding in *S. hermonthica* provides an explanation for some of the observed variation within populations in gross morphology (Musselman *et al.*, 1979) and seed coat ornamentation (Jones & Safa, 1982). It may also account for the development of a degree of race specificity in *S. hermonthica* in response to different host species (Wilson-Jones, 1955; King & Zummo, 1977), and to different varieties of those species (Doggett, 1965; Ramaiah, 1981). The development of various degrees of host-preference in local populations of the species throughout its range may be directly attributed to natural selection upon variability for the genetic factors determining the physiology of the parasite, variability which has been preserved in populations by outbreeding.

This propensity for developing new strains of the parasite, able to preferentially attack particular host species or varieties, casts doubt upon the effectiveness of the pot-techniques widely used for screening cereals for *Striga* resistance. These methods employ bulk samples of *S. hermonthica* seed which, we may confidently predict, are mixtures of a large number of genotypes. There is also an excess of *S. hermonthica* seed in the test pot but it is usual to find only a small proportion of the seed has attacked the cereal (Parker & Reid, 1979). Thus it is possible that apparently susceptible cereal varieties are resistant to some, possibly to many, of the genotypes of *S. hermonthica* present in a bulk seed sample. Their resistance is not detected because the plant succumbs to other genotypes of the parasite present in the mixture. Even sick-plot trials, because of the wide range of varieties of cereal species studied, will tend to favour the build-up of genetic diversity in the plot which, while not invalidating the empirical value of the trials, will obscure any genes present in the cereal which will afford protection against particular *Striga* genotypes. The isolation and characterization of host-specific genotypes in *S. hermonthica* should make it possible for the plant breeder to recognize genes in his cereal which offers degrees of protection against them. The method would be analogous to the present techniques for identifying genes offering protection against specific strains of fungal pathogens, and should be an improvement upon the present empirical techniques. Such work may provide an answer to the

important question of whether it will ever be possible to breed cereals acceptably resistant to *S. hermonthica*.

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Variation of Seed-coat Ornamentation in *Striga hermonthica* (Scrophulariaceae)

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ABSTRACT

The surface features of the seed of the parasitic flowering plant *Striga hermonthica* were examined with the scanning electron microscope. The details of ornamentation were constant on seeds from one plant but varied within and between populations and are probably due to out-breeding. The variation was not related to geographical origin or to host-preference. Testa patterns similar to those reported for other *Striga* species were found in the samples.

Key words: *Striga hermonthica*, Scrophulariaceae: Rhinanthoideae, hemi-parasite, testa, seed-coat, scanning electron microscope, genetic variation.

INTRODUCTION

Striga hermonthica (Del.) Benth. (Scrophulariaceae) is a hemi-parasitic weed of great economic importance in semi-arid African countries. It attacks the main grain crops, particularly maize, sorghum and *Pennisetum* millet, causing a serious reduction in yield and at times destroying the whole crop.

The existence of at least two physiological strains of *S. hermonthica* attacking *Pennisetum* millet or sorghum was reported by Wilson-Jones (1955) and confirmed by Parker and Reid (1979). The two strains are indistinguishable on the basis of seed-coat characteristics, having variable ornamentation in both strains, but some species (and species-complexes) can be distinguished (Musselman and Parker, 1981). A wide range of variation in plant size, corolla structure and colour within Nigerian populations was reported by Musselman *et al.* (1979).

In this paper we present evidence from the surface features of the testa of mature seeds of *Striga hermonthica* which indicates that populations of that species in Sudan and West Africa are variable with respect to the genes determining the ornamentation of the seed coat.

MATERIALS AND METHODS

Seeds of *S. hermonthica* were obtained from localities throughout the range of the species as detailed in Table 1. The seeds brought from Sudan were collected either from one capsule, 'single plants' or as a mixture, 'bulk collection', gathered from different plants of a single host species.

Seeds were mounted on stubs using double-sided adhesive Sellotape and coated with

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TABLE 1. *Location and host for* Striga hermonthica *seed collections examined in the present study*

Host plant species	Location and year of collection	Seed source
Pennisetum americanum	Gambia 1979	WRO
P. americanum	Sudan 1978	WRO
Sorghum bicolor	Nigeria 1977	WRO
S. bicolor	Gambia 1979	WRO
S. bicolor	Sudan, Shambat 1980	SS
S. bicolor	Sudan, Shambat 1979	KUFA
S. bicolor	Sudan, Abu Neaama 1980	KUFA
S. bicolor	Sudan, Nesheshiba 1980	SS
Sorghum sudanicum	Sudan, Nesheshiba 1980	SS

Key to source of seeds: KUFA, Khartoum University, Faculty of Agriculture (bulk collection); SS, Collected by one of the authors from a single capsule; WRO, Weed Research Organization, Oxford, UK (bulk collection).

45 nm of gold–palladium in a Polaron Cool sputter-coater and examined in a JEOL scanning electron microscope. Care was taken to record the surface features only on perfect, fully-developed seeds.

We are following Musselman and Mann (1976) in using the terminology of Chuang and Heckard (1972) for describing seed-coat ornamentation.

RESULTS

The size and shape of seeds and the prominent pattern of primary ridges on the surface varied considerably in all the collections examined. We noticed that the collections of seeds from single plants were more uniform with respect to other less obvious features and we concentrated our observations upon these features. These were: the density of protuberances on the primary ridges, on the secondary ridges and on the area in between them; the prominence of the rim on top of the primary ridges and the prominence of the secondary ridges. In contrast, these features varied considerably on seeds from bulk samples. Some seeds had a high density of protuberances all over the seed-coat [Fig. 1(a)] while others only had them on the primary and secondary ridges [Fig. 1(b)]. In a small number of seeds, protuberances were almost absent [Fig. 1(c)]. Indeed, a whole range of intermediates occurred between those seeds having a surface densely covered with protuberances and those which were almost smooth, in the same bulk sample [Fig. 1(d)].

The rim on top of the primary ribs also varied in prominence, sometimes being broad and high [Fig. 1(a)–(c)] sometimes being narrow and low [Fig. 2(a)]; both types are shown in [Fig. 1(d)]. The shallow secondary ridges, which run lengthwise or crosswise between the primary ridges may be prominent [Fig. 2(a), (b)] or obscure [Fig. 2(c), (d)].

This variation occurred irrespective of whether the collections were made from a sorghum or *Pennisetum* field. A similar range of variation was found in collections made

FIG. 1. Scanning electron micrographs of part of the testa of *Striga hermonthica* seed gathered from plants parasitizing sorghum in Sudan. (×700). (a) Seed from plant in field at Shambat. The sides of the ridges and the area between them are densely covered with protuberances. (b) Seed from a second plant in the same field. The few protuberances are confined to the sides of the ridges. (c) Seed from another plant in that field. The ridges and area between are almost smooth. (d) Seeds from a bulk collection from Abu Neaama. The surface of the upper seed is covered with protuberances; the lower seed has none.

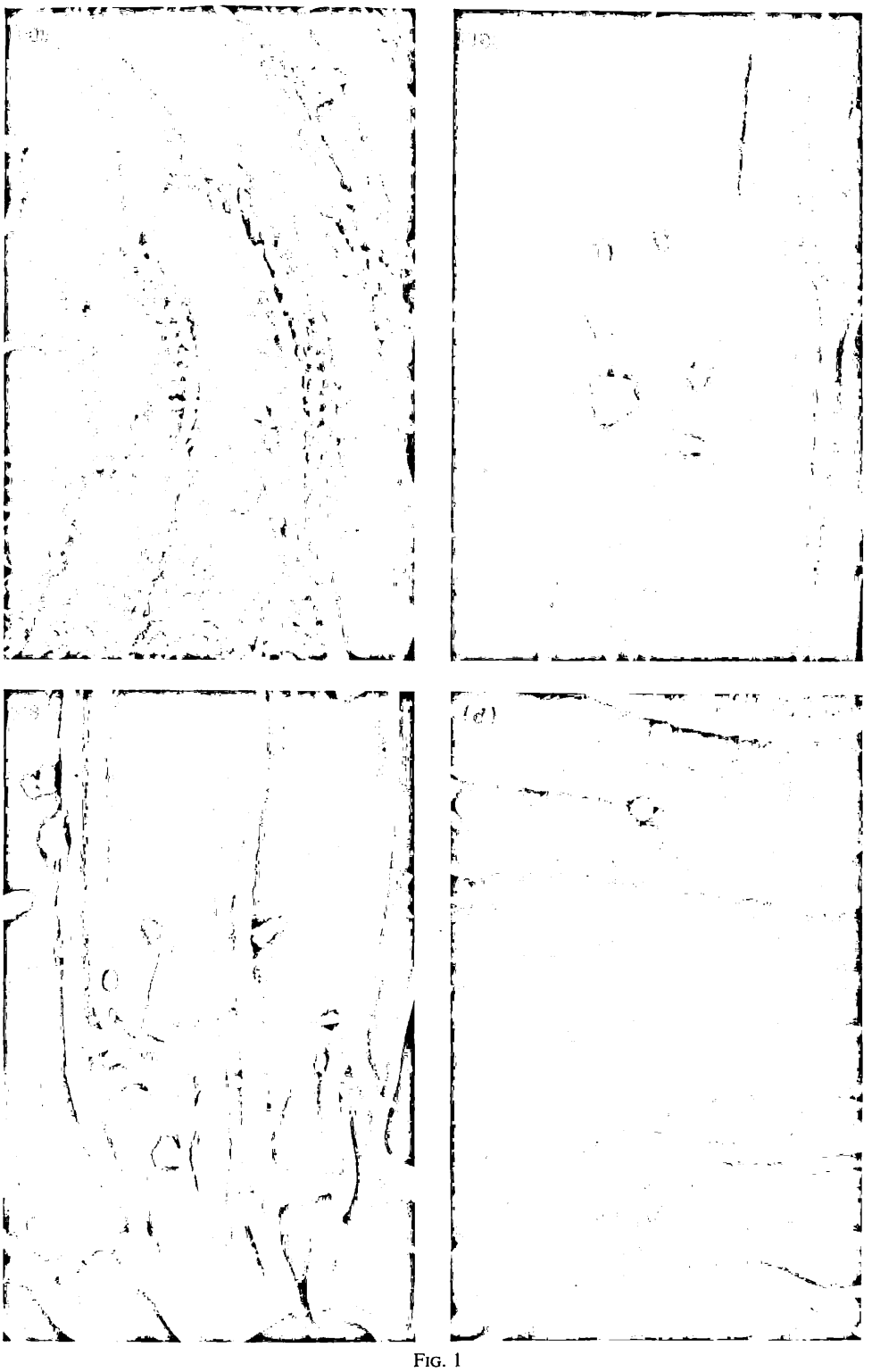

FIG. 1

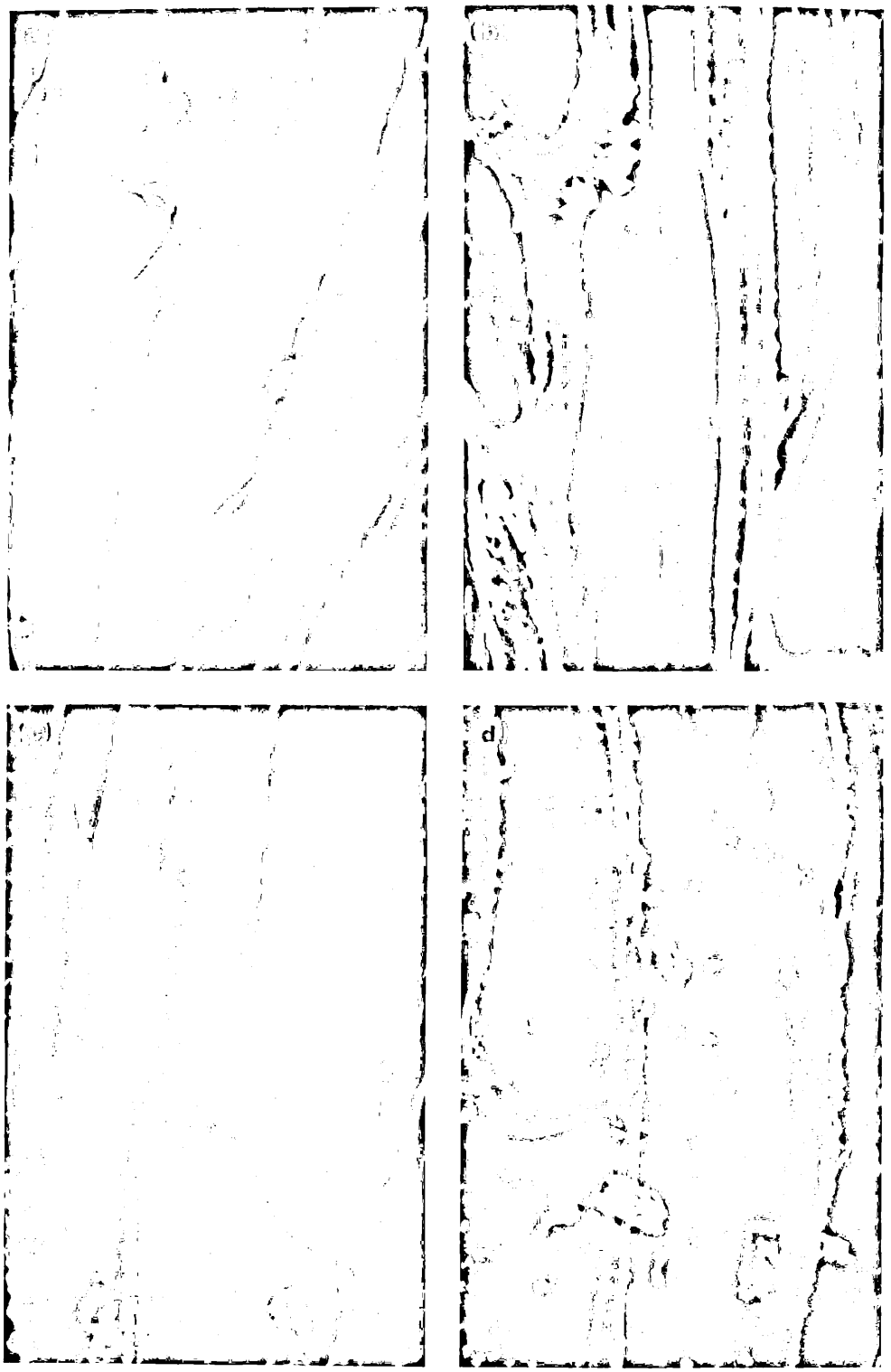

FIG. 2

in different countries. Because of this wide range of variation in all the bulk samples examined we selected a few representative types for illustrative purposes to represent all the collections.

DISCUSSION

Most of the obvious variations in shape, size and surface reticulation of *S. hermonthica* seeds did not seem to be directly under genetic control. Some of the smaller and misshapen seeds appeared to be empty, having presumably been stimulated to develop by the act of pollination but, because there were a large number of ovules in each pistil, some of them might not have been fertilized. The diversity of ornamentation detail in bulked seed collections contrasted with the similar ornamentation of seed gathered from one plant and indicates that the less obvious features of testa ornamentation are genetically determined.

The presence of a diversity of testa ornamentation within this one species, *S. hermonthica*, casts doubts upon the statement by Musselman and Parker (1981) that 'surface features of seeds [of *Striga* species] may have taxonomic value'. In our material we found ornamentation similar to those which they figure for *S. gesnerioides* [Fig. 2(a)], *S. asiatica* [Fig. 2(b)], *S. forbesii* [Fig. 2(c)] and *S. passargei* [Fig. 2(d)]. This conflicts with their conclusion that 'surface feature may be of value in the study of species complexes such as the *S. hermonthica*/*S. aspera*/*S. passargei* group'.

The discovery of a wide range of ornamentation detail not only in different populations but in characterizing different individuals within populations, implies considerable genetic variability for characteristics of the seed coat in *S. hermonthica*. In turn this variability indicates to us that the species is outbred. This conclusion agrees with the observations of variation of flower form and colour reported by Musselman *et al.* (1979). C. Parker noted frequent insect visitors to this species in Upper Volta (verbal communication) which could effect cross-pollination. Our own observations of the effectiveness of cross- and self-pollination indicates that the species is outbred (Jones and Safa, in preparation).

The demonstration of genetic diversity in the bulk collections of *Striga* seed which are used for testing cereal varieties for resistance to the parasite may account for the inconsistency of results obtained in such tests. Furthermore, cereal varieties resistant in one region are often susceptible in another: sometimes even in one region a variety does not perform consistently. This unpredictability of attack by *S. hermonthica* may be attributable to local variation in the genes determining host-specificity, due to outbreeding. It is certainly an additional complicating factor which must now be considered by the plant-breeder seeking to develop *Striga*-resistant cereal varieties in Africa.

ACKNOWLEDGEMENTS

We wish to thank the following who have contributed to the study: University of Gezira, Sudan for funding the trip to Sudan to collect seeds of *S. hermonthica*; Mr Chris Parker

FIG. 2. Scanning electron micrographs of part of the testa of *Striga hermonthica* seed gathered from plants parasitizing sorghum in Sudan (× 700). (a) Seed from a bulk collection from Shambat. The protuberances are confined to the ridges, as in Musselman and Parker's plate of *S. gesnerioides*. (b) Another seed from that collection. The surface detail resembles Musselman and Parker's plates of *S. asiatica*. (c) Seed from the Abu Neaama bulk collection. The elongated protuberances on the primary ridges and the smaller ones on the secondary ridges resemble those on Musselman and Mann's plate of *S. forbesii*. (d) Another seed from the Shambat bulk collection. The primary ridges have obscure, and the secondary ridges prominent protuberances as in Musselman and Parker's plate of *S. passargei*.

of the Weed Research Organization, Yarnton, Oxford, UK, for providing us with seeds and for his keen interest and advice; Dr Litton Musselman, Old Dominion University, Norfolk, Virginia, USA, for helpful discussions and encouragement; the Faculty of Agriculture, University of Khartoum, Sudan for permission to collect seeds from their experimental plots; Mrs Heather Hermes for technical advice with the scanning electron microscope.

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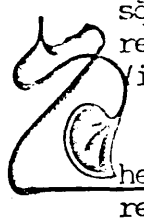
CONNECTION BETWEEN THE VASCULAR TISSUE OF STRIGA HERMONTHICA AND ITS HOST

The vascular tissues in the region of Striga hermonthica and its host, sorghum, were studied using fluorescence microscopy. Haustoria were fixed in formalin- acetic-alcohol (1:1:8) and cleared and softened in 1N NaOH for one hour in a water bath at 60°C; stained in a 0.1% aqueous solution of aniline blue dissolved in 0.1N K_3PO_4. The haustoria were gently squashed and examined through a fluorescent microscope, using blue light (incident) for exciting the dye.



Xylem elements in the roots of S. hermonthica and sorghum fluoresced reddish-yellow, due to their lignified cell walls, while the phloem fluoresced greenish-yellow, characteristic for callose-containing tissues. In the haustoria both types of fluorescence were observed and it was possible to follow the xylem and phloem of the parasite in the haustorium and to see their direct attachment to the xylem and phloem of the host root respectively.

The separate link between xylems and of phloems in the haustorial region supports Roger's and Nelson (1959) view of separate pathways for the translocation of organic matter and for the passage of water from host to parasite. It does not support Okonkwo's (1964) evidence in favor of a dual function of the xylem in S. hermonthica.

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