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PREFACE

These Proceedings include papers presented at the Third International Symposium on Parasitic Weeds held at the International Center for Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria, May 7-9, 1984.

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Third International Symposium on Parasitic Weeds, Aleppo, 1984.

INCIDENCE OF PARASITIC HIGHER PLANTS AND STRANGLING FIGS ON ORNAMENTAL TREES IN PENANG, MALAYSIA.

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Abstract. Twenty seven per cent of the 1295 trees representing 45 species at the Universiti Sains Malaysia campus were found to be infested mainly with *Loranthus ferrugineus* Jack (*Scurrula ferruginea* (Jack) Dans.), *L. pentandrus* Linn. (*Dendrophthoe pentandra* (Linn.) Miq. and the strangling fig, *Ficus retusa* Linn. The most susceptible trees were *Adenanthera pavonina, Artocarpus* sp., *Ceiba pentandra, Enterolobium saman, Grewia tomentosa, Lagerstroemia flos-regenae, Pongamia pinnata* and *Pterocarpus indicus*. Three species, *Casaurina equisetifolia, Jacaranda filicifolia* and *Plumeria obtusa* were probably resistant under local conditions to infestation by the parasitic higher plants and the strangling figs. The effects of these infestations on the aesthetics of trees and possible control methods are discussed.

INTRODUCTION

In developing countries such as those in South-East Asia, urban centres have been subjected to rapid population increases during the last twenty years due to migration of people from the rural areas. In the establishment of new urban centres or the enlargement of existing towns and cities, landscaping with trees plays an important role. Various types of trees were grown depending on their size, morphology, habit, reproductive properties and sites of planting. The island of Penang is situated at the north-west of Peninsular Malaysia. It is about 24 km long by 14.5 km wide. The climate is typically tropical with an almost even climate. The annual mean temperature is about 27°C and an annual rainfall of about 2440 mm spread fairly evenly during the year. Georgetown, the capital of the State of Penang, is situated on the north-east of the island. Extending from Georgetown towards the north-east and also south of the island are new suburbs with housing estates. About 15 km south of the Georgetown is the new urban centre of Bayan Baru. Tree planting is taking place in the suburbs as well as in the new urban centre. Many of the trees are young and this study of the incident of parasitic plants on older trees would indicate future action in control methods.

METHODS AND MATERIALS

The study site chosen is the 113 hectare campus of Universiti Sains Malaysia. The campus is on the eastern side of the island of Penang and is about 8 km south of Georgetown. The campus, a former military camp, is on rolling ground with hills and valleys. There are a great variety of trees, some occurring naturally, some planted before the establishment of the campus and others are being planted as and when physical development of the campus progresses. Tree planting as part of the landscaping of the University is co-ordinated by the University's Development Office. A map

of the campus was obtained and divided into grids. All trees above three metres height in each grid area were examined, first visually on the ground and then with a pair of binoculars for the presence or absence of parasitic plants. Although *Ficus* spp. are not parasitic in the true sense, they were included in the study since some of the hosts are eventually killed and the aesthetics of the hosts are badly affected. Specimens of parasitic plants were collected and identified using keys by Henderson (1959) and Ridley (1924). Their morphological characteristics were studied to enable identification through the binoculars. The host trees were identified using keys mainly by Corner (1952).

RESULTS

A total of 45 species of trees numbering 1295 individuals were examined for the presence of parasitic higher plants and strangling figs. 350 trees (or 27%) were found to be infested with parasitic higher plants and strangling figs (Table 1). Eight species, *Adenanthera pavonina*, *Artocarpus* sp., *Ceiba pentandra*, *Enterolobium saman*, *Grewia tomentosa*, *Lagerstroemia flos-reginae*, *Pongamia pinnata* and *Pterocarpus indicus* were found to be seriously (more than 40% individuals) infested. Fourteen other species were moderately (20-40%) infested. The rest were slightly infested or not infested. Most of the species without infestation were small in number and could have escape attack. However, three species, *Casuarina equisetifolia*, *Jacaranda filicifolia* and *Plumeria obtusa* with 40 or more individuals were not infested. These species are probably resistant to attack.

The most common parasitic higher plant is *Loranthus ferrugieus* Jack (*Scurrula ferrunginea* (Jack) Dans) which was found to attack 24 species of trees. *L. pentandrus* Linn. (*Dendrophthoe pentandra*(Linn.) Miq.) is also common but found on a smaller number of individual trees. In most cases both *L. ferrugineus* and *L. pentandrus* were found together on the same trees. *Viscum orientale* Willd. is not an important parasitic higher plant. *Ficus retusa* is the most important strangling fig and is found on almost all the tree species where parasitic higher plants were present.

DISCUSSION

One of the most important considerations for growing ornamental trees is their aesthetics. When trees are infested with parasitic higher plants, the appearance of the trees are affected. These parasitic higher plants cause stunting of young trees in Penang and heavy infestation could cause death of branches and delay in flowering, for example, *Lagerstroemia flos-reginae* infected by *Loranthus ferrugineus*. Many of the tree species in the University campus are also roadside trees. Infestation of ornamental trees also weaken the branches so much that there is a danger of falling branches during the many tropical rain storms. *Loranthus ferrungineus*, *L. pentandrus* and the strangling fig , *Ficus retusa* occur on a wide variety of host trees. These three species had also been reported to be common on lowland trees in Peninsular Malaysia and Singapore (Corner, 1952; Ridley,

1924). The loranthaceous plants have also been recorded as having a wide host range including trees of economic as well as aesthetic value (Johari and Bhatnagar, 1972). *L. pentandrus* was also found to be important in teak plantations (Nuhamara, 1976). Animals such as monkeys, squirrels and birds have been reported (Corner, 1952; Kuijt, 1969) to eat the fruits of mistletoes and strangling figs and deposit the seeds on the branches of trees where they germinate into bushes. Birds especially in the family Dicaeidae are also regarded as important pollinators of mistetoes (Kuijt, 1969). Pruning of branches of trees infested with parasitic higher plants have been carried out in Penang, Malaysia. However, the method is not effective. Chemical control may be the answer. The control of mistletoes by chemical methods has been satisfactorily practised in Australia. Sprays and injections by various chemicals particularly formulations of 2, 4-D have successfully killed *Amyema* and related genera growing mainly on *Eucalyptus* (Greenham and Brown, 1957). The control of *Loranthus* on teak by diquat was found to be satisfactory (George, 1966). However, there is the difficulty of reaching the parasitic higher plants with conventional spraying methods. The alternative is to look for a systemic selective herbicide to be applied to the roots of trees for control of the parasitic higher plants on the branches.

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Table 1

A list of tree species in Universiti Sains Malaysia (USM) Campus infested by parasitic higher plants and *Ficus* spp.

Nos.	Trees	No. infected/ total no. (**)	No. of trees infested*					
			L1	L2	V	F1	F2	F3
1.	*Acacia auricyliformis*	8/81	3	4	0	1	0	0
2.	*Achras zapota*	0/9			–			
3.	*Adenanthera pavonina*	10/23(++)	4	8	0	6	2	1
4.	*Anacardium occidentale*	1/9	0	0	0	0	1	0
5.	*Artocarpus heterophyllus*	2/18	1	1	0	0	0	0
6.	*Artocarpus integer*	1/5(+)	0	0	0	0	1	0
7.	*Artocarpus* sp.	6/12(++)	0	1	0	5	0	4
8.	*Averrhoa bilimbi*	0/3			–			
9.	*Canarium* sp.	8/35(+)	4	4	1	3	1	1
10.	*Cassia siamea*	0/2			–			
11.	*Casuarina equisetifolia*	0/65			–			
12.	*Ceiba pentandra*	3/5(++)	2	3	0	0	0	0
13.	*Cinnamomum iners*	0/1			–			
14.	*Crypteronia paniculata*	2/6(+)	0	0	1	2	0	0
15.	*Delonix regia*	35/99(+)	14	18	0	24	1	1
16.	*Durio zibethinus*	4/49	1	2	0	3	0	0
17.	*Enterolobium saman*	50/83(++)	38	38	3	9	0	15
18.	*Erythrina indica*	11/42(+)	1	1	0	6	2	6
19.	*Erythrina* sp.	1/4(+)	0	0	0	1	0	0
20.	*Eugenia grandis*	3/8(+)	2	1	0	0	0	0
21.	*Eugenia* sp.	6/21(+)	1	1	1	4	1	0
22.	*Fagraea fragrans*	0/2			–			
23.	*Garcinia mangostana*	6/27(+)	2	4	0	1	0	0

Nos.	Trees	No. infected/ total no.(**)	No. of trees infested*					
			L1	L2	V	F1	F2	F3
24.	*Grewia tomentosa*	71/107(++)	30	67	0	0	0	0
25.	*Jacaranda filicifolia*	0/46			-			
26.	*Lagerstroemia flos-reginae*	8/8(++)	4	8	0	2	0	0
27.	*Mallotus macrostachyus*	1/3(+)	0	0	0	1	0	1
28.	*Mangifera foetida*	0/2			-			
29.	*Mangifera* sp.	2/25	1	2	0	0	0	0
30.	*Melia indica*	4/41	0	2	0	2	0	1
31.	*Mimusops elengi*	0/1			-			
32.	*Morinda citrifolia*	35/107(+)	13	24	6	28	0	0
33.	*Moringa oleifera*	0/1			-			
34.	*Nephelium* sp.	1/47	0	1	0	0	0	0
35.	*Parkia javanica*	0/1			-			
36.	*Peltophorum pterocarpum*	24/80(+)	1	7	0	12	2	6
37.	*Pithecellobium dulce*	2/5(+)	2	1	0	0	0	0
38.	*Pithecellobium jiringa;*	0/1			-			
39.	*Plumeria obtusa*	0/40			-			
40	*Pongamia pinnata*	1/2(++)	1	1	0	1	0	0
41.	*Pterocarpus indicus*	30/60(++)	6	9	3	24	6	3
42.	*Spathodea campanulata*	1/8	0	0	0	1	0	0
43.	*Tamarindus indica*	7/31(+)	3	2	1	2	3	0
44.	*Terminalia catappa*	6/69	1	5	0	0	1	0
45.	*Vitex* sp.	0/1			-			
	TOTAL	350/1295	135	215	16	138	21	49

*L1 = *Loranthus pentandrus*; L2 = *L. ferrugineus*; V = *Viscum orientale*; F1 = *Ficus retusa*; F2 and F3 = *Ficus* sp. 2 and 3.

** ++ = >40%; + = 20-40%; the rest < 20%

Third International Symposium on Parasitic Weeds, Aleppo, 1984.

POSSIBLE HOST DEPENDENCY OF EPICORTICAL ROOT FORMATION IN CULTIVATED SCURRULA PULVERULENTA (LORANTHACEAE)

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Abstract. Two plants of the mistletoe Scurrula pulverulenta G. Don (Loranthaceae) were grown successfully from Indian seeds on Quercus ilex L. and Hedera helix L. The plant on ivy grew vigorously and after three years produced many flowers which matured normal berries and seeds. The plant on oak grew less vigorously and in the same period produced fewer flowers and berries; the seeds, nonetheless, developed normally. The plant on ivy, although it reached considerable size, did not produce epicortical roots, whereas the plant on oak developed a long strong epicortical root. Another plant on the same oak, which was accidentally broken and did not reach flowering, also developed a strong epicortical root. Two possible hypotheses are discussed - (1) the epicortical root formation in Scurrula pulverulenta may be genetically inhibited in some plants; (2) the inhibition may be a response to the nourishment available from the host tree.

INTRODUCTION

In my cultivation trials of mistletoes I had some success with Scurrula pulverulenta (Grazi, 1983). Bhojwani and Johri (1977) have described the aerial roots of this mistletoe in Dehra Dun. Two of my Scurrula pulverulenta plants which reached maturity behaved quite diffently in the production of aerial roots. Kuijt (1981) prefers to use the term epicortical roots and attaches great taxonomic importance to the presence or absence of such roots in neotropical mistletoes. I have found that some Scurrula pulverulenta plants mature without epicortical roots and propose explanations for this.

METHODS AND MATERIALS

Ripe fruits of Scurrula pulverulenta grown on orange tree at Shillong (India) were gently squashed soon after arrival in January 1979 and 36 sticky seeds were placed on 3 host plants (Citrus sp., Hedera helix, Quercus ilex) in pots in a temperate (10 - 25°C) greenhouse. Records of the germination and further development were supplemented with drawings and photographs.

RESULTS

Only 3 of the 36 seeds grew more than 10 cm. The remaining 33 either did not germinate or produced poor seedlings that died after 3 leaves had appeared.

One plant on ivy grew to 100 cm by 1982 and produced more than 100 flowers between October 1981 and March 1982. The berries were ripe by early spring 1982 and gave normal seeds, some of which developed when placed on new hosts (Grazi, 1983). Unfortunately this ivy was killed by drought in summer 1982 so that it was not possible to follow further development of the parasite.

One plant on oak grew less vigorously during the same period (40 cm in 1982) and gave only 19 flowers in February 1982. Only 2 berries ripened and these produced no plantlets. The next year the plant grew another 60 cm and flowered abundantly from October to March 1983, producing 49 berries. The seeds germinated and produced plantlets on several hosts.

Another plant on the same oak also grew slowly and was broken accidentally at the base in summer 1981. By that time it had reached 16 cm. After this accident it began at once to form a new shoot from the basal part, which reached 20 cm in 1983. This plant has not yet flowered.

Both mistletoe plants on oak produced a strong epicortical root more than 30 cm long, with several secondary haustoria and leafy buds. The plant on ivy, on the contrary, did not produce an epicortical root.

The 16 *Scurrula pulverulenta* plantlets of the new generation sown in 1982 have produced a total of 5 epicortical roots in 1983: 2 on *Quercus ilex*, none on *Hedera helix*, 3 on other hosts (Grazi, 1983).

DISCUSSION

Although more material is needed to reach a final conclusion, the present results indicate that in *Scurrula pulverulenta* the formation of epicortical roots may not be a constant feature. Bhojwani and Johri (1977) did not mention the absence of epicortical roots in their studies. Kuijt (1981) reported a probably exceptional case of a mature plant without epicortical roots in the loranthaceous *Oryctanthus occidentalis*. He considers the occurrence or absence of epicortical roots of great importance for systematic purposes.

If the occurrence of epicortical roots in Scurrula pulverulenta in India is a general rule and if these organs have a taxonomic value, it is interesting to discuss how a plant could reach maturity and considerable size without forming any such root.

(1) It may be that the plant on ivy was genetically different from the others. If so, genes inhibiting the epicortical roots must be present but seldom expressed in nature.

(2) It may be that the ivy had an inhibiting effect on epicortical root formation, but still provided sufficient nutrients for vigorous development.

To test further the two hypotheses a vegetative transplantation of Scurrula pulverulenta from oak to ivy is planned.

If the main function of epicortical roots is to increase the number of haustoria, then it is possible that host trees that provide abundant nutrients inhibit root development. If a certain area lacks such hosts, then all Scurrula pulverulenta growing there will produce epicortical roots.

On the other hand, if the function of the epicortical roots is mainly to increase vegetative reproduction, then it may be that plants with the genotype giving no epicortical roots are gradually eliminated from a natural population and reappear only in very exceptional circumstances.

I hope that the results of the second and third sowings may bring further arguments for one or the other of the hypotheses.

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Third International Symposium on Parasitic Weeds, Aleppo 1984.

COMPARATIVE HISTO-PHYSIOLOGICAL STUDY OF THE GERMINATION AND ESTABLISHMENT OF THREE MAJOR PARASITES : *VISCUM, OROBANCHE* AND *CUSCUTA*

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Abstract. The damage caused by the genera *Viscum, Cuscuta* and *Orobanche* is so severe that all possibilities of control must be considered. The autotrophic period of their life, i.e. their germination and their establishment on a host, seems to be the best period to apply any method of control. In this paper, morphological and cyto-physiological aspects of the germination and of the establishment of the three pests are compared.

Organs developed during germination show different behaviour. For *Orobanche* and *Cuscuta* the organ in the radicular position does not present the typical organization of a root. Its cells neither divide nor synthesize nuclear DNA ; they are blocked in the G_1 or G_2 phase of their cycle. The growth of this organ is only due to auxesis. On the contrary the cells of the hypocotyl developed by *Viscum* and those of the stem and of the shoot apex of *Cuscuta* show a nuclear reactivation after a resting period. The nature of this reactivation is reported. The growth of these organs involves meresis and auxesis.

This cyto-physiological work brings new insights into the biology of these parasites which should be helpful for a future chemical program of control.

INTRODUCTION

Viscum, Orobanche and *Cuscuta* are the major parasitic flowering plants widespread in Europe and all around the Mediterranean sea. They cause important damage on trees for *Viscum*, on cultivated plants for *Orobanche* and *Cuscuta*.

Many aspects of the biology of these three pests have been reviewed in recent literature (Gill and Hawksworth, 1961 ; Kuijt, 1969, 1977 ; Kuijt and Toth, 1976 ; Ozenda and Capdepon, 1979 ; Pieterse, 1979 ; Musselman, 1980 ; Frochot and Sallé, 1980 ; Sallé, 1975, 1977, 1983). These parasites show many differences related to their organization, ecology and physiology.

The control of these plants is a problem still to be solved. Whatever the method of control, i.e. biological, chemical, mechanical or use of resistant crop varieties, it is fundamental to know the biological mechanisms involved.

The aim of this paper is to present and to compare cyto-physiological data concerning the germination and the establishment of these three major parasites. Beside the fact that these plants cause important damage in agri-

cultural areas, three other reasons justify such a comparative work : (1) *Orobanche* and *Cuscuta* are holoparasites, whereas *Viscum* is an hemiparasite, (2) two plants are stem parasites (*Viscum* and *Cuscuta*) and the third is a root parasite (*Orobanche*), (3) *Viscum* and *Cuscuta* start their germination without the presence of a host whereas *Orobanche* needs the secretion of a stimulus by the host root system.

A better knowledge of the morphological and cyto-physiological events occurring during the germination and the establishment of the parasite on the host should indicate the stages where the parasites are the most sensitive to treatment and consequently facilitate the choice of an efficient herbicide.

METHODS AND MATERIALS

Germination was obtained under appropriate conditions.

Seeds of *Viscum album* L., collected in February, were sterilized by a sodium hypochlorite solution and then placed in Petri dishes on a wet filter paper. The germination occurred at 23°C under a 16 h. photoperiod. 1, 2, 3, 4, 10 and 20 day-old seedlings were fixed in formalin and prepared for the cytological study. For histoautoradiography, samples were treated as described earlier (Sallé, 1975, 1977). The establishment of *Viscum album* was studied on experimental implantations on host branches (apple tree).

For broomrape, the minute seeds of *Orobanche crenata* Forsk. collected by Aber in Morocco, were inserted between two sheets of filter paper on which *Vicia faba* (var. *major*) seeds were placed. During germination of the latter the root exudate provoked the germination of the parasite. Two samples of "procaulômes" and young plants on the host were collected under a binocular. One was treated by the Cohen and Garner's method (1971) for SEM observation, the other for cytological work.

For the germination of dodder, seeds of *Cuscuta lupuliformis* Krocker were treated with sulfuric acid for 10 minutes (Gaertner, 1950) and then rinsed in cool water. After a 3 h. imbibition, they were placed in crystallising dishes on a layer of wet filter paper under controlled conditions (20-23°C, 16 h. photoperiod). Samples were collected at intervals and treated for different purposes (SEM, light microscopy, autoradiography).

RESULTS

Organization of the embryos

Table 1 summarizes the most striking features of the embryos of the parasites. It is noteworthy that among these three genera, the embryo of *Orobanche* is the least structured. So, the polarity suggested by some authors (Krenner, 1958 ; Aber and Sallé, 1983) is very slight. During the embryo development of broomrape and of dodder the suspensor disappears very soon (Tiagi, 1951 a, b ; Johri and Tiagi, 1952).

Table 1

Comparison of some features of the embryos of *Viscum*, *Cuscuta* and *Orobanche*

Genera \ Feature	Cotyledon	Differentiated shoot apex	Differentiated organ in the micropylar zone	Endosperm	Chlorophyll
Viscum	2	+	+	+++	++
Cuscuta	0	+	+	++	0
Orobanche	0	0	0	+	0

Morphological aspects of the germination and establishment of the parasites

1) *Viscum*. During the germination of *Viscum* the hypocotyl elongates (Fig. 1) until its swollen tip reaches the host surface. Then it flattens against it and formes a holdfast (Fig. 2). The seedling soon stands erect after depletion of the starchy endosperm.

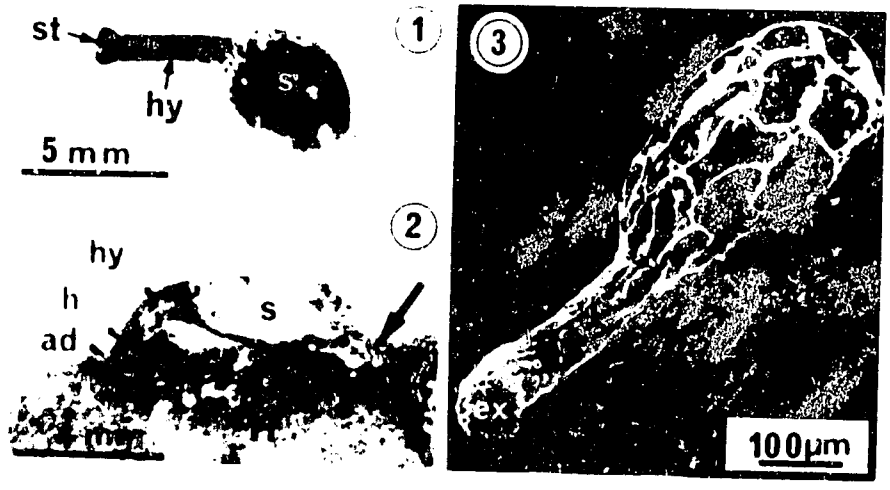

Fig. 1 - *V. album* : 20 day-old seedling with elongated hypocotyl, hy, and its swollen tip, st. s, seed.

Fig. 2 - *V. album* : early fixation on the host, H. ad, adhesive disc ; h, holdfast ; hy, hypocotyl ; s, seed. Arrow, dried viscid tissue.

Fig. 3 - *O. crenata* : well developed "procaulôme", Pr. ex, extremity ; s, seed.

2) *Orobanche*. During the germination of *O. crenata* seeds, stimulated by the root exudate, the cells of the micropylar zone expand out of the testa and form a filamentous organ (Fig. 3) which has been called a radicle (Chabrolin, 1939 ; Roger, 1954), a germ (Krenner, 1958), a "germ tube like" (Kadry and Tewfic, 1956 ; Abou-Raya *et al.*, 1973) or also a "procaulôme" (Velenovsky, 1905 ; Cézard, 1976 ; Aber and Sallé, 1983). It can elongate

to 3 or 4 mm. When it reaches a host root it differentiates papillae and some of its elements penetrate the host tissues rapidly.

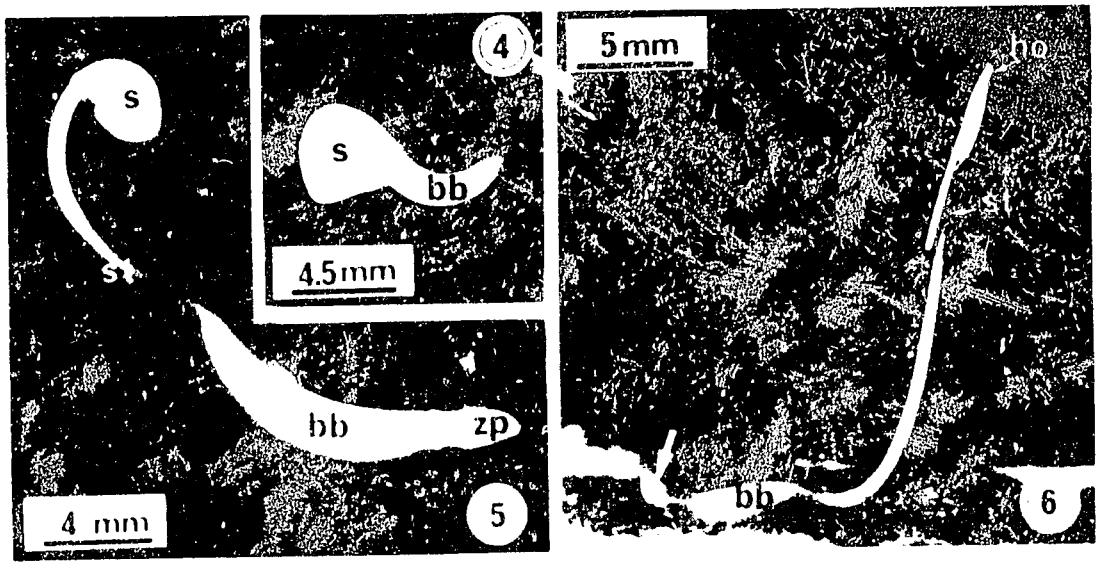

Fig. 4 to 6 - *C. lupuliformis* at different stages.

Fig. 4 - Stage B, note the curvature of the future basal body, bb.

Fig. 5 - Stage D, the zone of papillae, zp, is well developed at the tip of the basal body, bb. The extremity of the stem is still enclosed in the seed, s.

Fig. 6 - Stage E, the tip of the basal body is necrosed, arrow. The stem, st, elongates and forms a hook, ho.

3) *Cuscuta*. In *Cuscuta* one finds the most complicated type of germination. Recently, in our laboratory, phenological stages of germination have been demonstrated for *Cuscuta lupuliformis* by SEM (Le Coz, 1983).

During stage A the part of the embryo located in the micropylar region protrudes at the surface. Stage B is characterized by the curvature of the "radicular organ" (Fig. 4). Then it elongates, gains an orange pigmentation and differentiates papillae at its tip. During stage D (Fig. 5), it reaches its maximum length and width whereas the elongation of the stem continues, its tip still enclosed within the endosperm. During stage E (Fig. 6), the swollen basal part of the seedling degenerates while the endosperm is torn out and the tip of the stem formes a hook. Then the necrosis of the "radicular organ" increases, whereas the hook opens and the stem begins to circumnutate actively to bring it into contact with a suitable host.

These observations show that the organ formed during the germination of *Viscum* and *Orobanche* has a well-defined purpose : to establish a contact with the host surface. On the other hand, the basal body of the dodder forms a mechanical base for the seedling and provides it with water and

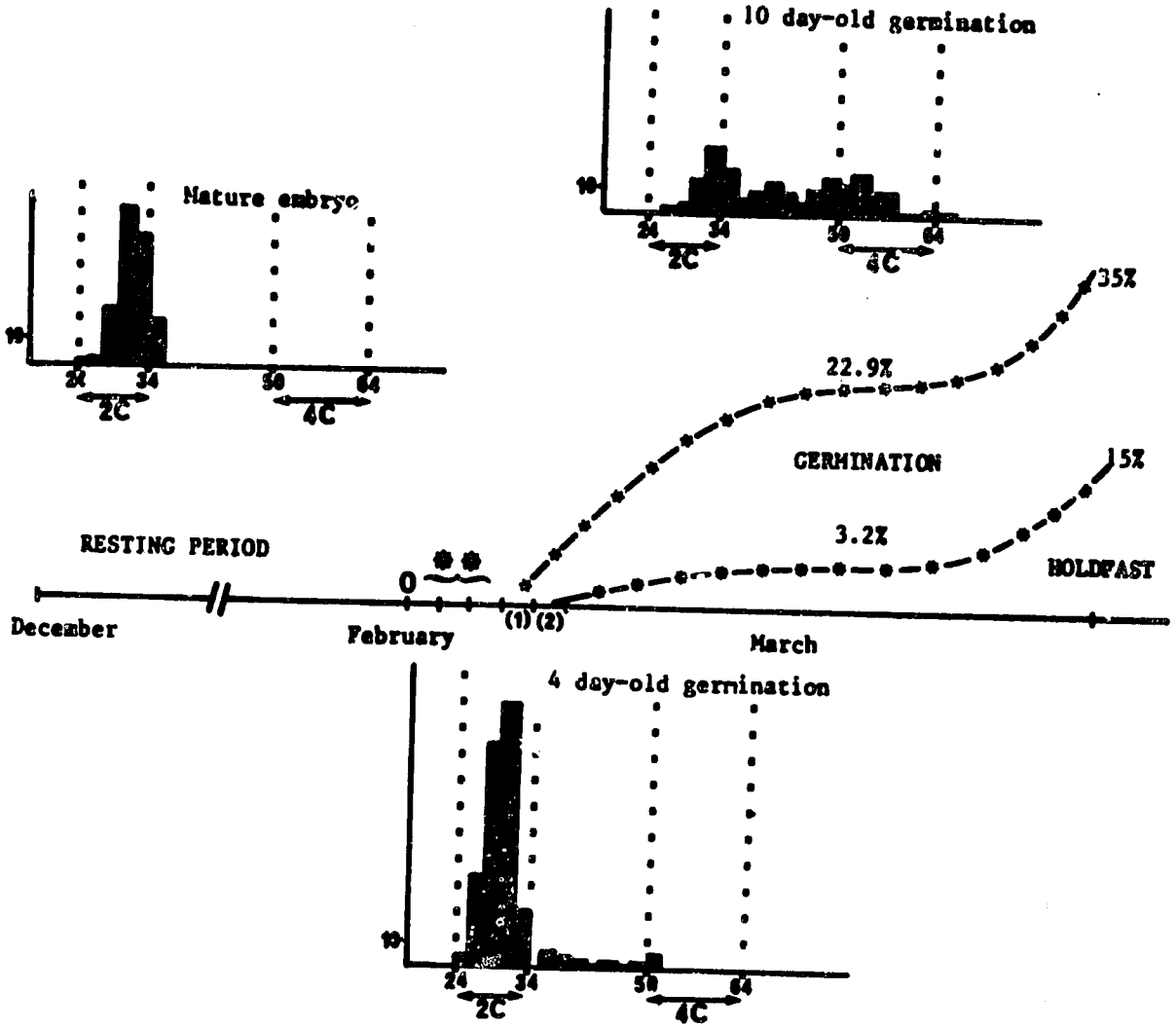

Fig. 11 - Nuclear events occurring during the germination and the establishment of *Viscum album*. ——*——, percentages of labelled nuclei ; —*— , percentages of mitosis ; ✱✱ , auxesis only ; (1), first labelled nuclei ; (2), first mitosis. The diagrams correspond to the cytophotometric measures.

mineral salts from the outset of germination. Broomrape is characterized by the weakness of the "procaulôme".

Comparison of the cyto-physiological aspects of the germination of *Viscum*, *Cuscuta* and *Orobanche*

From a cyto-physiological point of view the events occurring during the germination of *Viscum* are the best known among the three parasites considered.

1) *Viscum*. During the germination of *Viscum* the growth of the hypocotyl is mainly due to meristematic activity located in its extremity (Fig. 7). Sallé (1975) showed that these cells had meristematic characters, i.e. isodiametric shape, voluminous nucleus, poorly developed vacuoles and high density of cytoplasmic ribosomes. In mature embryos these cells do not synthesize DNA and never divide (Sallé, 1976). When the seeds are given good conditions for germination the hypocotyl elongates during the first 72 hours ; auxesis is clearly the only phenomenon involved in this hypocotyl elongation.

Two days after the beginning of germination the first labelled nuclei (after a 6 hours contact with a thymidine ^3H solution) were observed, and one day later the first mitosis could be detected (Fig. 11). Labelling and mitotic indices, calculated for 20 meristematic zones of 4 and 10 day-old germinations, showed a gradual increase of their values which remained at about the same level (22.9 % and 3.2 %) during the whole germination.

A cytophotometric study (Fig. 11) performed on Feulgen stained nuclei showed that, during the resting period, the nuclei of these meristematic cells were blocked in the G_1 synthetic phase of their cycle. After 4 days of germination a high percentage of the nuclei had 2C DNA content but some of them had higher values. Later on the DNA values extended over both the 2C and 4C ranges.

During the differentiation of the holdfast (Fig. 8), under the adhesive disc, the meristematic zone occupied the central zone, surrounded by parenchymatous margins. Labelling and mitotic indices increased respectively, from 22.9 % to 35.0 % and from 3.2 % to 15.2 %. These variations could be explained by a more restricted localization of the meristematic activity in the "coin de pénétration", perhaps associated with an acceleration of the cell cycle.

2) *Cuscuta*. Some former works (Mirande, 1900 ; Fritsche *et al.*, 1959 ; Truscott, 1966) and a very recent one (Le Coz, 1983) showed that the basal part of the seedling of *Cuscuta* did not present the typical organization of a root (Fig. 9). Parenchymas (cortical and medullary) were well developed, whereas the meristematic zone and the cap were missing. The cyto-physiological work of Le Coz demonstrated that during germination the basal and the upper parts of the seedling had different behaviour. They have been defined during the five phenological stages of germination. They are summarized in table 2.

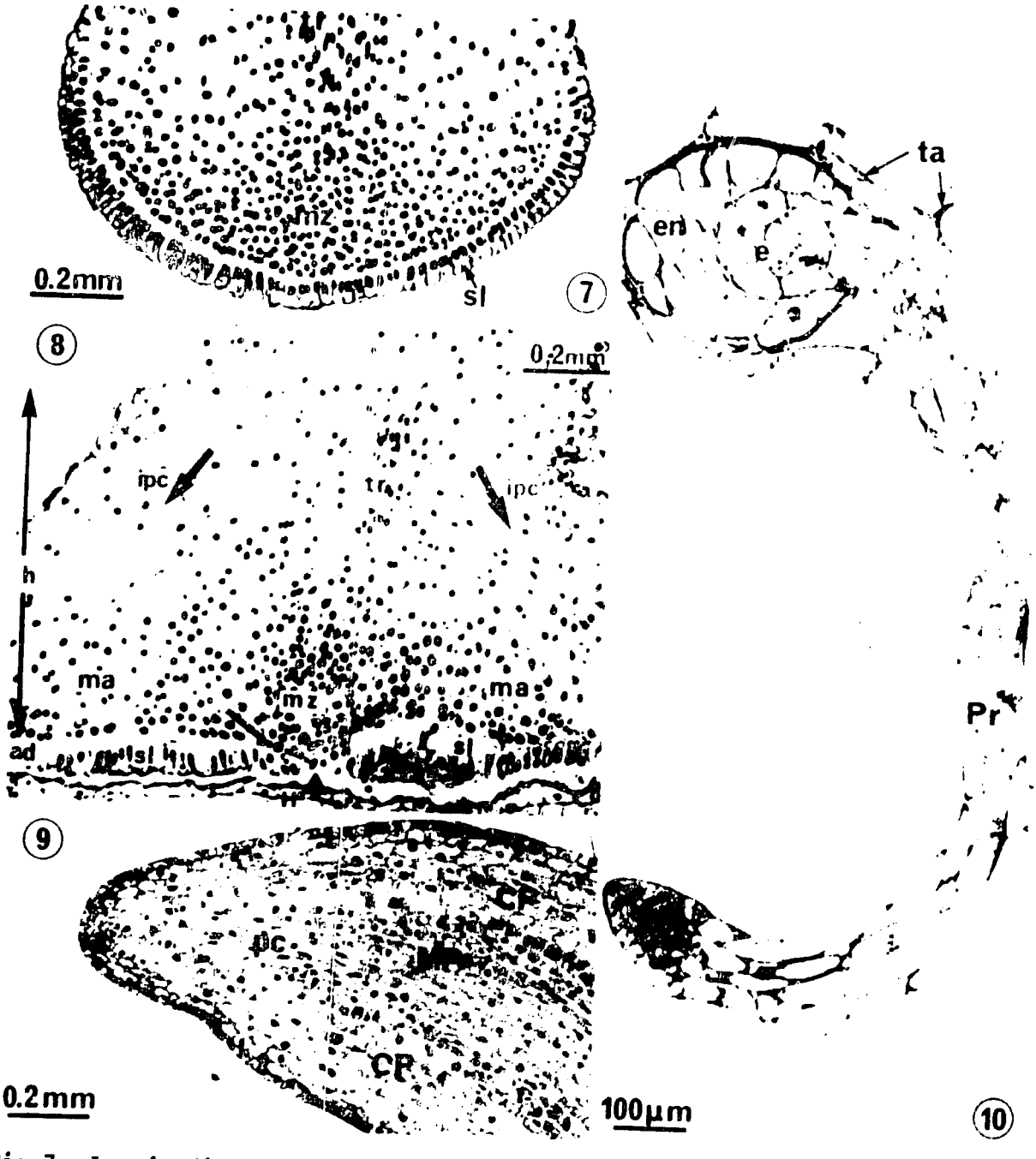

Fig. 7 - Longitudinal section of the tip of a growing hypocotyl of *V. album*. mz, meristematic zone ; sl, superficial layer ; tr, tracheid.

Fig. 8 - Longitudinal section of a holdfast, h, of *V. album*. ad, adhesive disc ; ipc, inner dead parenchymatous cells ; ma, margin ; mz, meristematic zone ; sl, superficial layer. Arrows show dead superficial cells.

Fig. 9 - Longitudinal section of the tip of the basal body of *C. lupuliformis* at the stage B. CP, cortical parenchyma ; MP, medullary parenchyma ; pc, procambium

Fig. 10 - Longitudinal of a germination of *O. crenata* with its "procaulôme", Pr, and its active cells, ex. e, rest of the embryo ; en, endosperm ; ta, testa.

Table 2

Cyto-physiological results obtained on *Cuscuta lupuliformis* from the imbibition to the beginning of the necrosis of the basal part of the seedling.

Nuclear DNA synthesis		Mitotic activity		Size of the cells of the basal part (µm)		Hydration and evolution of the substances stored in vacuoles (proteins) and in cytoplasm (starch)		phenological stages
bb	st m	bb	st m	L	W	bb	st	
-	-	-	-	40	22	numerous small vacuoles proteins +++ starch ++	very beginning of the hydration proteins +++ starch ++	A
-	-	-	-	X	X	1 to 3 medium sized vacuoles proteins ++ starch ++	vacuolation proteins ± starch ++	B
-	+++	-	+ only in st	102	35	one single large vacuole proteins ± starch ±	vacuolation proteins X starch +++	C
-	+++	-	++	110	54	single large vacuole proteins 0 amidon 0	single large vacuole proteins X starch +++	D
-	X	-	+++	X	X	necrosis of the cells	single large vacuole proteins X starch +++	E

bb, basal body ; m, meristem ; st, stem ; L, length ; W, width.
X, absence of results.

Whatever the phenological stage the basal part of the seedling neither divides nor synthesizes nuclear DNA. The cells show a marked enlargment, their length increasing from 40 µm (stage A) to 110 µm (stage D), whereas their width increases from 22 µm (stage A) to 54 µm (stage D). This enlargement is accompanied by vacuolation and depletion of stored material (proteins and starch). This differentiation ends with a total necrosis of the organ. These original results demonstrate that the growth of the basal part of the seedling is due only to significant auxesis.

During stages A and B cells of the shoot apex and of the stem neither synthesize nuclear DNA nor divide. As for the cells of the basal part of the seedling they are blocked either in the presynthetic phase G_1 or in the post-synthetic phase G_2 of their cell cycle. Only a cytophotometric study could determine the phase in which they are blocked. They reactivate during stage C, with a slight delay for the meristem. It is important to note that the parenchymatous cells of the stem show an intense nuclear activity. The newly formed cells vacuolate progressively with a delay as compared to the basal part of the seedling. Whereas proteins were hydrolized, starch was stored in the cells of the stem. We clearly demonstrated that the seedling growth involves meresis and auxesis in the shoot apex and also in the stem.

3) *Orobanche*. The weakness of the "procaulôme" explains why only few studies have been performed on it. Aber (1981) and Aber and Sailé (1983) never observed mitosis in the very active cells of the tip of the procaulôme (Fig. 10). Moreover, they noticed the important auxesis occurring in the parenchymatous cells. In a very long "procaulôme," they can reach 200 µm in length. One should note (Fig. 10) that the "procaulôme" was devoid of any conducting tissues ; its only function is to bring the terminal active cells in contact with a future host root ; its feeding role should not be very important. In *Orobanche* the shoot apex is initiated very late, after the differentiation of the tubercle.

DISCUSSION

The three parasites *Viscum*, *Orobanche* and *Cuscuta* differ from one another in their development, their location on the host and also in their cyto-physiological behaviour.

The radicular pole of the embryo always gives rise to a peculiar organ which is very different for the three examples. Organs of *Viscum* and *Orobanche* developed during germination show an extreme morphological dissimilarity. However, they look alike when they bring the active zone of the parasite in contact with the host plant. The situation is completely different for the basal part of the seedling of *Cuscuta* which plays both a mechanical and a fundamental feeding role. It taps water from the environment and supplies the upper part. Our cytological observations (highly vacuolated cells) corroborate the physiological work of Fer (1979) which shows the active role of the basal part of the seedling in its water supply. The life of this organ is very short (8 to 10 days). If the aerial part does not establish a functional connexion with the host tissues within this period, the progressive necrosis of the organ causes the death of the seedling.

The autotrophic period of *Viscum* is the longest (it can last several months) because the seed and the embryo are chlorophyllous. They produce most of the food necessary for the seedling.

Althoughmany experiments have been made with synthetic germination stimulants (Johnson *et al.*, 1976) or analogues (Saghir, 1979) or with herbicides (Dawson, 1979 ; Schmitt *et al.*, 1978 ; Gimesi, 1979 ; Schlüter and Aber, 1980 ; Mesa-Garcia and Garcia-Torres, 1982) new investigations need to be done in these ways to control parasitic plant development.

The autotrophic period of life of parasitic flowering plants represents the best moment to apply a chemical method of control. Already the present cyto-physiological data and the better knowledge of the development of these three parasites should suggest some herbicides able to perturb one of the growing phases. For example, herbicides inhibiting auxesis may be active only on the growth of the basal part of *Cuscuta* seedling or the "procaulôme" of *Orobanche*. It is evident that the weakness of the latter should be an interesting aspect although, so far, nobody has proposed any efficient action against it. On the other hand, herbicides affecting the mitosis or the microtubular system might have an inhibitory effect on the activity of the tip of the *Viscum* hypocotyl or on the stem development of *Cuscuta*.

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MECHANISMS OF RESISTANCE OF FOUR CULTIVARS OF POPLAR AGAINST *VISCUM ALBUM* L.

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Abstract. The genus *Viscum* is widespread in Europe, Africa and Asia. Economic losses caused by mistletoe are evident, but have never been estimated seriously.

Removal of aerial shoots and pruning are quite inefficient. Treatments with synthetic hormones or growth substances can give efficient results but they are not definitive and often they have to be repeated. The selection of resistant varieties of trees against mistletoe has appeared to be a promising and a complementary method of control.

Since 1974, the resistance of 4 cultivars of poplar, "Fritzi Pauley", "I_{214}", "Bergerac" and "Blanc de Garonne", has been investigated in France. The first is very susceptible whereas the last is resistant. The two others are intermediate. A histo-cytological study, undertaken in 1980, demonstrated that two barriers were involved in the resistance of poplar : a mechanical one and a chemical one. The mechanical barrier concerns the thickness of the phellem and the location of lignified fibers. The bark of resistant trees has a thicker phellem and more numerous and more superficial fibers. Moreover, there are anatomical features specific to each variety. The chemical one, involving an important secretion of polyphenols, is stimulated in response to the attack of the parasite.

In future, resistance to mistletoe infection should be taken into account before recommending new varieties of trees.

INTRODUCTION

Viscum album L. is a widespread parasitic flowering plant which parasitizes various kinds of trees, including orchard trees (apple, almond, pear), forest trees (fir, pine) and hardwood trees (poplar, maple, linden, etc.). Its development causes a general decrease in all parameters of production (Peter-Comtesse, 1940 ; Guinier, 1934 ; Gill and Hawksworth, 1961 ; Kuijt, 1969 ; Preston, 1977) : growth in height and in diameter, quality of the wood, setting of fruits and seeds. It can also provoke premature death of the host. Moreover, an increase in the susceptibility of the attacked trees to other pathogens is observed. For all these reasons, control of the european mistletoe becomes a serious problem.

Now, it is well established that the removal of aerial shoots is quite inefficient because the endophytic system regenerates new shoots rapidly (Sallé, 1977).

During the 1970's, some methods were assayed to eradicate *Viscum album* on fir using chemical treatments. For example, Delabraze and Lanier (1972)

sprayed various phytohormones and growth substances (2,4-D ; 2,4-MCPB ; 2, 4, 5-T ; chloro-2 ethyl phosphonic acid) on the foliage of mistletoe or injected them into the host trunks. Six months later they could observe the yellowing or even the death of the aerial part of the parasite. The efficiency of the treatments was assessed five years after their application (Frochot and Delabraze, 1979). Results depended on the nature of the substances, the methods and time of application. In some cases, a durable effect was observed for 2,4-MCPB and 2, 4, 5-T when sprayed on host foliage during the spring (April) or injected into the trunk at the end of the summer (September). In other cases a declining efficiency followed by a mistletoe recovery occurred one to three years after the application. Even if these treatments were tolerated by fir because of the selectivity of 2,4-MCPB and 2, 4, 5-T for this species, they are hazardous for hardwood trees. In such cases the substance must be sprayed only on the aerial parts of the mistletoe. In fact, they only destroyed the foliage of the parasite, leaving alive the endophytic system (Sallé, 1977) which is able to give rise to new aerial shoots the following years.

This is the reason why the selection of resistant varieties of trees appeared to be a promising and a complementary method of control against mistletoe. A new era began in 1978 with the work of Frochot *et al.* which showed by experimental inoculations differences in the susceptibility of 4 cultivars of poplar : "Fritzi Pauley", "I_{214}", "Blanc de Garonne" and "Bergerac".

To explain the mechanism of resistance against mistletoe, new artificial inoculations have been carried out on a wider scale and a histo-cytological study has been done.

METHODS AND MATERIAL

Experimental inoculations

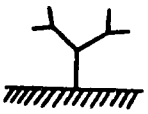
Experimental inoculations were made in a plot of the INRA Champenoux (Meurthe et Moselle, France) on a hundred 5 year old poplars belonging to 4 cultivars : *Populus trichocarpa* Torrey and Gray cv. "Fritzi Pauley" (FPL); *Populus* x *euramericana* (Dode) Guinier cv. "Bergerac" (BRG); *Populus* x *euramericana* (Dode) Guinier cv."I_{214}" and *Populus nigra* L. cv. "Blanc de Garonne" (BDG).

During the winter of 1979 ripe mistletoe berries were collected from infected poplars in the region of Nancy. Seeds from which the skin had been removed were placed on pieces of tarlatan 5 by 5. In April 1979 approximatively one thousand seeds were placed on trunks of each cultivar measuring 5 to 6 cm in diameter. Three months later, the tarlatan was removed with care. The development of the mistletoe was followed regularly according to phenological stages defined by Frochot *et al.* (1978), the histological characteristics of which had been specified in 1980 (Frochot and Sallé). The phenological stages and their corresponding histological features are summarized in Table 1.

Complete evaluation of the development of the mistletoe seedlings were performed 5, 19 and 41 months after inoculation. For the histological study samples of control and parasitized poplars were collected after 19 months.

Table 1

Histological characteristics of the different phenological stages

	Phenological stages	Histological stages
	(+) beginning of germination	growth of the hypocotyl with a free extremity
	(a) development of the holdfast	contact between the young holdfast and the host bark
	(b) holdfast well developed, endosperm disappeared	very beginning of penetration of the young primary haustorium
	(c) first two leaves	development of the primary haustorium and establishment of direct connexions between the two xylem tissues
	(d) and following : development of the following internodes	

Histological techniques

Freshly collected samples were fixed with formalin, then dehydrated in ethanol embedded in celloidin and sectioned at 60 µm thickness with a Tetrander Reichert microtome. The sections were observed unstained or stained with a methyleneblue-ruthenium red solution. Some cytochemical tests, such as periodic acid-Schiff (PAS) for polysaccharides, HCl-phloroglucinol for lignified cell walls, were used. Polyphenolic substances were characterized by their natural brownish colour and by a dark brownish staining obtained after fixing by Regaud's technique including a postchromisation.

RESULTS

Table 2 summarizes the behaviour of mistletoe berries 5 and 19 months after the beginning of the experiments.

After 5 months, many seedlings reached the phenological stages (+) and (a), i.e. they were germinating. In all cultivars, the percentages of germination were very high (FPL : 69.7% ; I_{214} : 60.6% ; BRG : 70.1% ; BDG : 81.3%) and were much higher than the natural percentage assessed at 10% by Frochot *et al.* (1978).

Table 2

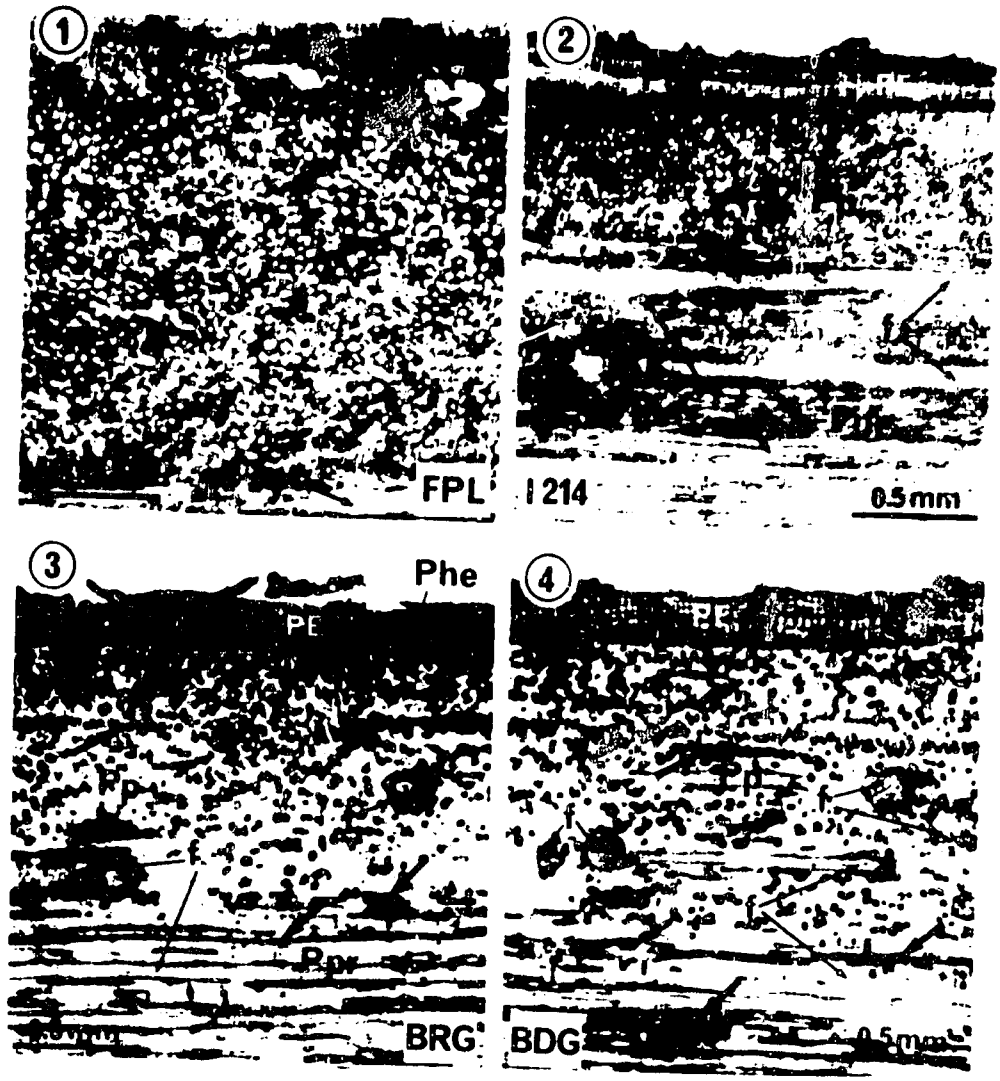
Behaviour of mistletoe berries on trunks of the 4 cultivars of poplar, 5 and 19 months after infection

cv	Seeds per inoculation \ Stages	+ 5 months (+) and (a)	(b)	+ 19 months (c)	(d)	(e)
FPL	1008	703	431	107	5	3
I214	960	582	505	5	2	0
BRG	768	547	194	6	0	0
BDG	768	625	157	0	0	0

19 months after inoculation differences appeared among the cultivars. Considering the percentage of living seedlings two patterns could be distinguished : the cvs "Fritzi Pauley" and "I214" showed a high percentage of live seedlings (respectively 54.1 and 53.3), whereas the cvs "Bergerac" and "Blanc de Garonne" had lower percentages (respectively 22.1 and 20.4). More interesting conclusions were obtained when taking into account the different phenological stages for each cultivar. On "Fritzi Pauley" and "I214", many seedlings reached the stage (b) (respectively 431 and 505) and even the stage (c) on the former (107). On the contrary, on the cvs "Bergerac" and "Blanc de Garonne", very few seedlings reached the stage (b) (respectively 194 and 157) and none reached the (c) stage on cv. "Blanc de Garonne". Between the 5th and the 19th months, the decrease in the number of seedlings reaching stage (b) and older ones can be explained by the death of many seedlings, still present on the trunks of the cvs "Bergerac" and "Blanc de Garonne".

A third estimate of the success or failure of mistletoe was performed 41 months after the inoculation. Percentages of seedlings reaching (c) and older stages were :"FPL" : 24.0% ; "I214" : 2.2% ; "BRG" : 1.5% and "BDG" : 0%. These percentages were undervalued because they were calculated after collection of several samples for the histo-cytological study. However, they clearly indicated that "Fritzi Pauley" was very susceptible whereas "Blanc de Garonne" was resistant ; "I214" and "Bergerac" were intermediate with a low level of successful implantations.

Before studying the mechanism of resistance of poplar to mistletoe, the histo-cytological organization of control trunks of the 4 cultivars was established. The same tissues were always present (Figs 1 to 4) : from the surface to the xylem, phellem, phellogen with thin phelloderm, secondary phloem, cambium and xylem were found successively. However, the comparison of longitudinal radial sections of healthy trunks of the 4 cultivars (Figs 1



Figs 1 to 4 - Longitudinal radial sections of trunks of the four cultivars. f, fiber ; Phe, phellem ; Pp, secondary phloem parenchyma ; Ppr, secondary phloem ray ; PE, periderm. Arrows show cells with a polyphenolic content.

to 4) showed important structural differences between the most dissimilar cultivars in respect to the resistance against mistletoe, i.e. "Fritzi Pauley" and "Blanc de Garonne". The cv. "Fritzi Pauley" showed thinner phellem (see, Figs 1 and 4, Phe). Moreover, its secondary phloem parenchyma was quite homogenous, whereas in the cv. "Blanc de Garonne", most cells had a brownish content of polyphenolic nature (Fig. 4, arrows). Fibers, f, were much more numerous in this cultivar. It is interesting to notice that all these anatomical aspects were specific for each cultivar. Cvs "I214" and "Bergerac" had an intermediate structure.

Figs 5 to 8 - Longitudinal radial sections of poplar trunks parasitized by mistletoe. The penetration of the primary haustorium, HAU, separates the phellem, Phe, into two layers : an external one, Phe.e. and an inner one, Phe.i.. f, fiber ; h, holdfast ; Pp, secondary phloem parenchyma ; Ppr, secondary phloem ray ; ✱ , polyphenols. Arrow shows cells with a polyphenolic content.

In order to determine the consequences of the penetration of the young primary haustorium into the host bark and to compare them among the 4 cultivars longitudinal radial sections were made on samples including the host bark, the holdfast and the young endophytic system. Figures 5 to 8 show that whatever the cultivar the penetration of the mistletoe provoked (1) a division into two parts of the phellem, Phe.e. and Phe.i., the inner part, Phe.i., enclosing the young primary haustorium, (2) a perihaustorial secretion of polyphenols, ✱ . However, some differences are obvious. For the cv. "Fritzi Pauley", in all samples investigated, the young primary haustorium passed rapidly through the inner phellem and the polyphenolic zone. On the contrary barks of cv. "Blanc de Garonne" (Fig. 8) reacted more intensively to the parasitic attack : the inner phellem was not broken by the young primary haustorium and the polyphenolic secretion was much greater. Moreover, in the "Blanc de Garonne", in the zone where the phellem breaks into two layers (Fig. 8, ✱) and along the inner phellem enclosing the haustorium,

the walls of the newly produced cells were stained by the test HCl-phloroglucinol, indicating a lignification of the walls.

It is important to keep in mind that samples were collected 19 months after inoculation. At this time mistletoe was in the stage (b). The parasitic tissues looked healthy on the cultivar "Fritzi Pauley", whereas they were often dead in cultivar "Blanc de Garonne".

So, in the 2 cultivars "Fritzi Pauley" and "Blanc de Garonne" the response of the host to the mistletoe attack was actually very different. For the two other cultivars, "I214" and "Bergerac", the intensity of the reaction varied from one sample to another according to their own resistance level. These different responses were very useful for the cytologist because the sequence of the events could be determined more easily. For instance, in some samples of the cultivar "I214" or "Bergerac" (Figs 6, 7), the delayed death of the primary haustorium illustrates the role of barrier assumed by clusters of fibers. In this case, the tip of the haustorium was blocked by these obstacles for some time before modifying its direction in order to continue grow.

DISCUSSION

These artificial inoculations on poplars belonging to cultivars "Fritzi Pauley", "I214", "Bergerac" and "Blanc de Garonne" inoculated with more numerous seeds and using a more sophisticated system, confirm the differences of susceptibility against mistletoe shown by Frochot *et al.* (1978). "Fritzi Pauley" is the most susceptible whereas "Blanc de Garonne" is completely resistant. The two other cultivars "I214" and "Bergerac" are intermediate, but rather resistant. This is true for poplars grown in the east part of France.

In all cases the germination of mistletoe occurred at a high level : 69% for "Fritzi Pauley", 60% for "I214", 70% for "Bergerac" and 81% for "Blanc de Garonne". Taking into account all our experimental conditions, the differences observed between the extreme percentages, i.e. 60% and 81%, could not be attributed either to an effect of the bark of the different cultivar on germination or to the influence of external factors (weather, predators, etc.). In no case, did seeds placed on the surface of poplar provoke a canker as described by Laurent (1899), Heinricher (1917) or Paine (1950) on pear branches. So, absence of healthy mistletoe on some clones of poplars did not correspond to a condition of "hypersensitivity" provoked by a toxin from the mesocarp of the berries.

From the present work some mechanisms of the resistance against mistletoe became evident from a cytological point of view. At the surface of the trunks of all cultivars the seeds germinated and the tip of their hypocotyl developed a massive holdfast which fixed them to the host. In these holdfasts the "coin de pénétration" (Sallé, 1975) always developed. During the stage (a), the behaviour of the germination was similar on all cultivars. Differences appeared during the stage (b) which was the most important because it marked the end of the autotrophic period of the biological cycle of mistletoe and the beginning of the parasitic one.

Irrespective of the cultivar, the reaction of the host to the parasitic attack consisted of (1) a separation of the phellem into two layers, the

inner part surrounding the young primary haustorium ; (2) a secretion of polyphenols in a perihaustorial zone ; (3) a lignification of the cell walls all around the sinker. However, the intensity of these three reactions varied according to the resistance of the host. In the susceptible cultivar "Fritzi Pauley" these structures were rapidly passed through by the young haustorium. It is interesting to notice that this cultivar had the thinnest phellem. Some individuals of this cultivar showed a very slight reaction reminiscent of that previously demonstrated by Sallé (1975, 1977) on apple branches. On the contrary, in the resistant trees (cv. "Blanc de Garonne") the haustorium was never able to disrupt these barriers. Moreover, the secretion of polyphenols and the lignification of cell walls were much greater. Our investigations showed that, even though all samples were collected 19 months after inoculation, most mistletoe grown on "Fritzi Pauley" was well developed. Connexions were established between the xylem of the host and of the parasite and also initiation of cortical strands was observed. These observations suggest the importance of the strength of the developing haustorium. In a sample some individuals may be stronger than others. So far it is impossible to know if it is a problem of the meristematic activity provoking the mechanical penetration or a problem of the quality (or the quantity) of enzymes involved in a questionable enzymatic penetration.

In conclusion, for cytologists, 4 factors at least are implied in the phenomenon of resistance of poplars against mistletoe : (1) the thickness of the phellem ; (2) the development of an inner phellem ; (3) the secretion of polyphenols ; (4) the lignification of the newly formed cell walls. It is evident that the thickness of the phellem represents a mechanical barrier ; the thicker the phellem is, the more resistant the tree is. The involvment of the three other factors is not so evident even though they have often been mentioned during the attack of other pathogens (fungi, nematodes). Presently, these reactions of barks of the four cultivar to mistletoe are compared to those resulting from a mechanical wounding produced by a needle. This study should indicate if only a living organism is able to induce these cytological events. If so the implicationof an elicitor-phytoalexin system will be open to consideration.

This work brings new insights into the understanding of host-parasite relationships. Even though many points concerning the phenomenon of resistance are still unclear, we hope that it will stimulate interest in further research.

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ECOLOGICAL STUDIES OF CISTANCHE TUBULOSA WIGHT.

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Abstract. Cistanche tubulosa Wight, an obligate phanerogamic root parasite, is of common occurrence in desert tract of Rajasthan, NW India. It has been found in two colour variants, viz. pale yellow and lilac. On sand dunes and sandy plains the parasite is seen flowering from January to March with a preference for Aerva persica Merrill, Calligonum polygonoides Linn and Calotropis procera R. Br. as hosts while on river bed terraces it is found to flower from August to October with a preference for Salvadora oleoides Dcne and S. persica Linn. as the hosts without any observable damaging effects. No monocot or annual dicot plant species are seen to host this parasite. The subterranean portion of C. tubulosa, except in the haustorial root attachment regions, degenerates and decomposes soon after seed setting. The parasite perennates through a coralloid mass of adventitious branches of the remaining subterranean portion.

INTRODUCTION

Cistanche tubulosa Wight (Orobanchaceae), an obligate phanerogamic root parasite, is the only Cistanche species reported from the Indian subcontinent (Hooker, 1885, Sabnis, 1941). It parasitizes a wide variety of indigenous xerophytic plants growing on sand dunes, sandy plains and river bed terraces in arid and semi-arid zones of Rajasthan.

The present investigation, was undertaken to study some of the ecological aspects, viz. host range, distribution and phenology of this parasitic species in Western Rajasthan.

DESIGN OF FIELD STUDIES

Excursions were undertaken in the months of August, October, January and March and the observations were recorded for the years 1978 and 1979. The trips were arranged in such a way as to cover most of the areas in Western Rajasthan, viz. Bikaner, Phalodi, Pokaran, Jodhpur, Barmer, Jaisalmer, Ajmer, Sikar and Churu. Host plants were recorded only after tracing the haustorial attachment region in the soil. Specimens of C. tubulosa, with root attachment of the host intact, were preserved and deposited in the Department of Botany, Dungar College, Bikaner. The distribution pattern and the phenological characteristics of the parasite were recorded.

OBSERVATIONS AND DISCUSSION

A. Host range

A consolidated list of host plants of C. tubulosa based on the records available and the author's work is presented in Table 1. The parasite has been recorded on 35 plant species, belonging to 14 families of flowering plants. In Western Rajasthan, it has been reported by the author to occur on 8 plant species (Table 2). Acacia jacquemontii Benth. had not previously been recorded as a host. It is interesting to note that host species are all perennial dicots with the exceptions of Paspalidium (Jafri, 1976) and some grasses (Gray, fide Nairne, 1976), which are monocots. During the present survey the author has not found C. tubulosa on any monocot or any annual dicot plant species even though such plants were growing close to the parasite which was present in abundance on Salvadora oleoides Decne. and S. persica Linn. along the boundaries of cultivated fields on river bed terraces.

Table 1. A list of hosts of Cistanche tubulosa Wight.

Host plant	References
Acacia	Graham, 1957; Tiagi, 1952.
Acacia etbaica Scweinf	Sir G. Taylor, 1965*.
Acacia jacquemontii Benth	Agrawal, 1980.
Aerva persica Merrill	Satyanarayan, Saxena and Gaur, 1966; Agrawal, 1980.
Aerva pseudotomentosa Blatt.and Hall.	Qadir, 1965.
Arthrocnemum indicum Moq.	Qadir, Ahmed and Qureshi, 1966.
Atriplex	Graham, 1957.
Atriplex helimus R. Br.	Sir G. Taylor, 1965*.
Atriplex stocksii Boiss.	Qadir, Ahmed and Qureshi, 1966.
Boerhaavia	Sir G. Taylor, 1965*.
Calotropis gigantia Br.?	Khan, 1961.
Calotropis procera R. Br.	Kashyap, 1936; Anvery, 1954; Ghani, 1957; Hasanain and Rehman, 1957; Chaudhri and Qadir, 1958; Gray (fide Nairne, 1976); Sharma and Tiagi, 1979; Ratnam, 1978 (In lit.); Agrawal, 1980.
Calligonum polygonoides L.	Ghani, 1957; Khan, 1961; Agrawal, 1980.
Capparis decidua (Forsk) Edgew (Or apphyla Roth).	Blatter and Hallberg, 1918-21; Khan, 1961; Yogi, 1970 (in lit).

Table 1 - contd...

Host plant	References
Capparis spinosa L.	Graham, 1957; Sir G. Taylor, 1965*.
Commiphora sp.	Graham, 1957.
Haloxylon	Post, 1937; Tackholm, 1956.
Lycium	Post, 1933; Tackholm, 1956.
Lycium barbarum L.?	Blatter and Hallberg, 1918-21.
Mimosa hamata Wild ?	Blatter and Hallberg, 1918-21.
Panderia pilosa	Ghani, 1957.
Paspalidium sp.	Jafri, 1976.
Pemphis	Graham, 1957.
Pemphia acidula Forest	Sir G. Taylor, 1965*.
Retma	Post, 1933; Tackholm, 1956.
Salsola	Kochar, 1958.
Salvadora oleoides Decne	Khan, 1961; Agrawal, 1980.
Salvadora persica Linn.	Khan, 1961.
Suaeda fruticosa Forsk.	Kashyap, 1936.
Suaeda maritima Dumort ?	Qadir, Ahmed and Qureshi, 1966.
Suaeda monoica Forsk	Chaudhry and Qadir, 1958.
Suaeda nudiflora Mog.	Chaudhry and Qadir, 1958.
Tamarix dioca Roxb.	Ahmed, 1961.
Tamarix nilotica ?	Graham, 1957.
Zygophyllum album ?	Graham, 1957.

* Fide Qadir, et al. (1966).
? Doubtful.

Table 2. Host range and distribution of C. tubulosa in Western Rajasthan.

Locality	Host
BIKANER	Calligonum polygonoides, Calotropis procera and Aerva persica
NAGAUR	Calotropis procera and Calligonum polygonoides
CHURU	Calotropis procera
SRI GANGANAGAR	Calotropis procera
JODHPUR - Banar	Calotropis procera
Luni	Calotropis procera and Acacia jacquemontii
Phalodi (river bed terraces)	Calotropis procera and Aerva persica

Table 2 contd...

Locality	Host
JAISALMER	Salvadora oleoides, and Calotropis procera
JALORE	Salvadora oleoides and S. persica
BARMER	Calotropis procera
AJMER	Calotropis procera
SIKAR	Calotropis procera

B. Distribution

The parasite is restricted to certain arid and semi-arid zones of Africa and Asia. There is no report from comparable arid zones in America, Europe or Australia. In Africa it has been reported from a narrow belt extending from Morocco to Egypt along the Mediterranean coastal region, from Ethiopia in the north to Tanganyka in the south, though Socotra, Somalia and Kenya along the Eastern coast, and in Senegal on its Western coast. In Asia it is reported to occur abundantly all over Arabia, Syria, Iraq and Iran in the west, extending to Pakistan, the Western parts of India, Afganistan, and Turkmanistan in the east.

In Rajasthan, the author found this parasite in the arid and semi-arid regions of Jaisalmer, Barmer, Bikaner, Churu, Nagaur, Sikar, Ajmer, Jodhpur, Pali and Jalore districts. The parasite was seen on sand dunes, sandy plains, and on river bed terraces. It tended to grow profusely along the sides of roads, rail tracts, and near villages but was less abundant in more remote places. It was observed to grow closely around host plants and up to as far as the host roots were likely to spread. The actively growing roots (secondary and tertiary) seemed to be the most favourable for attachment of the parasite, the latter being found rarely on the primary roots.

As C. tubulosa occurs on a wide variety of hosts growing in various types of xeric habitats, it seems to be a fairly versatile parasite. However, it has been found that it may not necessarily grow on the same host in ecologically different regions, e.g., it has been found to grow profusely on xerophytic plants like Aerva persica Merrill, Calligonum polygonoides Linn, and Calotropis procera R. Br., on sand dunes and sandy plains in Bikaner, Barmer, Jaisalmer, Churu,

Sikar, Sri Ganganagar, Jaipur and Nagaur but it may not attack all these hosts when present on river bed terraces (reported to have saline soils) in Jalore and Sanchore regions where it is restricted mainly to Salvadora oleoides and S. persica (Table 2). This indicates that certain ecological factors might determine whether C. tubulosa would grow on a particular host in a particular region or not. Further work is required to determine the factors responsible for such a behaviour of the parasite.

C. Phenology

C. tubulosa is a scapigerous herb. It makes its appearance in later winter months, particularly in December and January, when the flowering axes (scapes) emerge from the ground. The emergence of the scapes continues till about the middle of February. By this time most of the scapes come to full bloom. The flowering scapes, being spikes, continue to produce flowers in an acropetal succession for about a month, and then by the end of March they winter. On Salvadora species growing on river bed terraces the parasite makes its appearance during August - September, and comes to bloom by the end of October and withers by the end of December.

The subterranean portion of the parasite runs horizontally as a rhizome for a short length of 2 - 35 cm from its haustorial attachment, then becomes erect as subterranean vertical axis and finally emerges from the soil as a flowering axis. The subterranean portion is about 2 - 8 cm in diameter. It is succulent and its fully turgid cells are densely packed with starch grains of various shapes and sizes. Several scapes, usually 2 - 12, may arise from the same haustorial attachment. All of them do not develop simultaneously but emerge one by one so that while one scape may be in flower the others may be in various pre-flowering phases of development. The whole plant is covered with spirally arranged triangular scales which are densely crowded in the aerial portion; in the subterranean portion scales are few and distantly placed. The scales may be either pale yellow or lilac. The scales of the aerial portion of the stem bear flowering buds in their axils and behave as bracts. The spikes are 12 - 74 cm long and 2 - 7 cm in diameter. They are unbranched, usually have 10 furrows, appear turgid and become dry after seed-setting.

Each spike is densely covered by numerous flowers (240 - 300). Development of flowers is in acropetal succession. Elongation of the spike ceases soon after the formation of fruits in the basal region. A spike continues to elongate and produce flowers for about 8 - 10 days; fruit formation and maturation requires about 5 - 8 days. The

flowers may be pale-yellow or lilac in colour, irrespective of the host species parasitized. However, flowers of only one colour were noticed on the scapes arising from the same haustorial attachment. It was further noticed that the aerial as well as subterranean stem axis was dull lilac if the flowers were lilac, and pale if the flowers were pale yellow. How the flower colour correlates with that of the stem is under investigation by the author.

Being a total root parasite, C. tubulosa is dependent for all its nutritional requirements on the host plant. It could also depend upon the host for the supply of some hormones or their precursors for the initiation of its flowering. However, observations showed that the parasite flowered variously before, simultaneously with or after the flowering of the host and no generalisation appears possible.

Mature fruits (capsules) are dark grey in colour, 2 - 4 cm long and 1.0 - 1.5 cm in diameter. They are ovoid, oblong, laterally compressed and beaked. Each capsule contains numerous (1000 - 5000) seeds which are dark grey in colour. Capsules dehisced anterio-posteriorly along two furrows seen on pericarp. After dissemination of seeds by wind the dry spike remains as a barren axis. Seeds are small (1.25 mm in diameter) but larger than those of Orobanche species, subglobose and reticulately punctuated.

There was no obvious damaging effect of the parasite on the host. This may partly be because of the short life span of the parasite and partly because of the optimum growth and development of the host during this period. However, it needs experimental confirmation. The parasite seemed to wither within 25 - 30 days of its appearance above the ground. The aerial portion shrank and dried and the subterranean portion was found to undergo degeneration, emitting a foul odour. In the case of the parasite on Salvadora species in the Jalore region, where it was possible to trace the haustorial attachment, it was found that the parasite did not disappear completely, but was present in the form of a coralloid mass of dormant adventitious buds attached to the host roots (Fig. 1). This suggests that the parasite could probably perennate through these dormant buds, which could emerge and produce scapes under favourable conditions. Although the host-parasite attachment regions could not be traced in the later periods on Aerva persica and Calligonum polygonoides on account of their extensive root system in the sandy soils, yet the possibility of the existence of such a perennation mechanism cannot be ruled out. This contention received some support by the author's incidental observation of such coralloid mass of adventitious buds on Calligonum polygonoides (Fig.2). This feature may be of special ecological significance as it

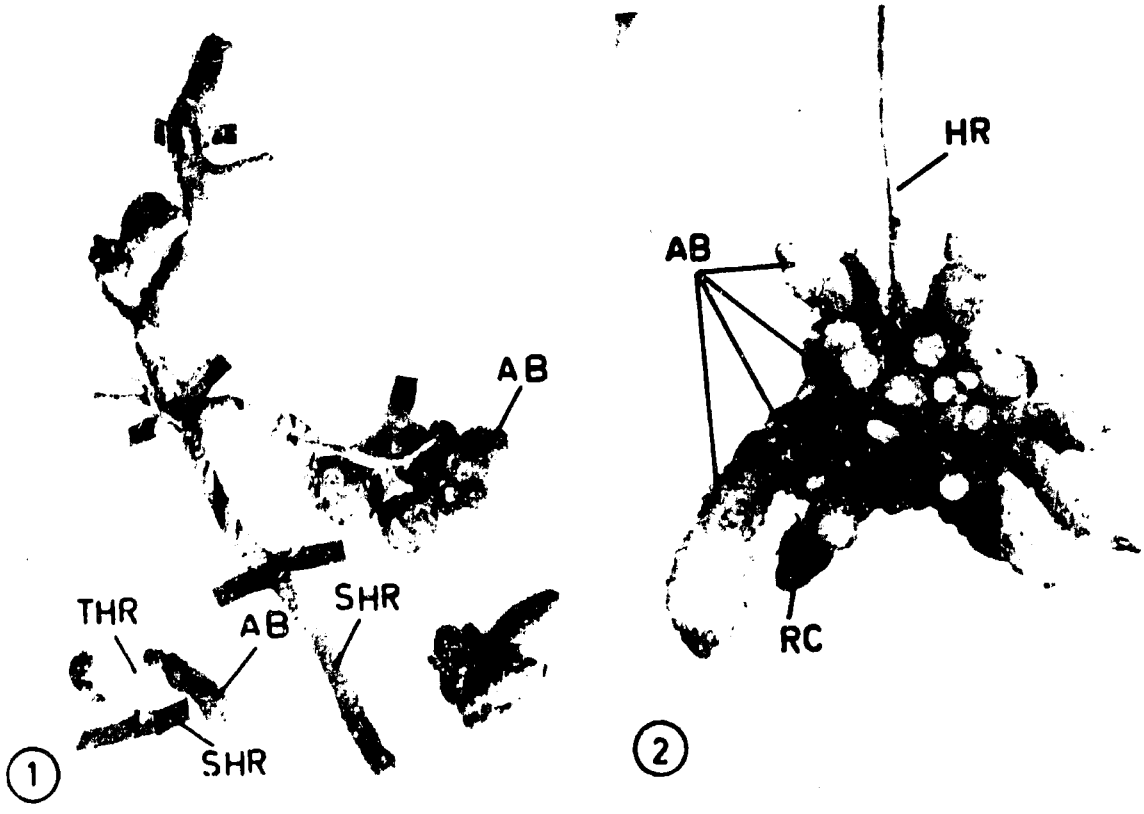

Fig. 1 - 2: Coralloid masses of adventitious buds (or branches).
Fig .1.Parasite on Salvadora oleoides X 1/5.
Fig. 2.Parasite on Calligonum polygonoides X 1/6.
(AB, adventitious buds/branches; HR, host roots; RC, root cap; SHR, secondary host roots; THR, tertiary host roots).

indicates the probability of the establishment of the perennial habit in the parasite. Rechinger (1964) has also considered this as a perennial parasite. However, further work is needed for establishing this aspect. This coralloid mass further appears to show the presence of a root cap on one of its adventitious branches (Fig. 2) indicating that some of them may behave as roots extending to other host roots and producing secondary suckers. This contention is supported by similar observations by Skene (1938) in Orobanche.

C. tubulosa may exist in two different ecological forms. This conjecture is based on the morphological differences observed in coralloid masses as aforesaid, i.e. the adventitious branches of the parasite on Calligonum are cylindrical (Fig. 2) while those on Salvadora are almost bulbous (Fig. 1). The difference in the time of emergence and flowering of the parasite on these two hosts growing in different habitats also supports this contention. Similarly, Qadir et al. (1966) noted this difference in the emergence and the time of flowering and stated that C. tubulosa in N. Pakistan growing on halophytes flowered in October to February while in S. Pakistan on non-halophytes it flowered in March to May indicating the probability of the existence of ecological races of this parasitic species. Further study is planned to investigate this problem.

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OROBANCHE RAMOSA IN THE UNITED STATES

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Orobanche ramosa L. is a holoparasitic weed which causes damage to many important agricultural crops. Primary hosts are members of the Solanaceae, Asteraceae, Brassicaceae, and Fabaceace (Musselman and Sand, 1982). This species of Orobanche is considered a "noxious weed" in the U.S.A. in that it was determined to be a threat to agronomically important crops. These conclusions were based on host specificity tests conducted to determine crop susceptibility. Our research indicated that the Texas strain of O. ramosa can parasitize tomato (Lycopersicum esculentum L.), tobacco (Nicotiana tabacum L.), sunflower (Helianthus annuus L.), bell pepper (Capsicum frutescens L.), bellbean (Vicia Faba L.), and celery (Apium graveolens L.), (Coffin, Musselman, and Eplee, 1983). Flax (Linum usitatissimum L.) was found to be an excellent inducer of seed germination, but did not function as a host. From these studies we concluded this newly found population of O. ramosa to be of sufficient economic importance to warrant the implementation of an eradication effort. Therefore, the eradication program was initiated against this parasitic noxious weed.

In 1928 O. ramosa was discovered in California on tomatoes where it has remained a minor but recurring problem. This has been the only infestation of O. ramosa in the United States of agronomic importance. In February 1981, an infestation of O. ramosa was discovered in Karnes County, South Central Texas growing on deciduous weeds along the rights-of-way of the public highway (Musselman and Nixon, 1981). This Texas strain appears different in appearance from the California strain. Even though this infestation was in a non-cropped area, the potential for loss by crops susceptible to O. ramosa justified the undertaking of investigations to ascertain the agronomic importance of this Texas strain.

Regulatory procedures have been implemented to prevent further dissemination of Orobanche from the infested area. An intensive survey program will ensure that all areas of infestation are located. The infestation has been found to occur only on about 240 ha along some 80 Km of highway rights-of-way. There are only a few sites where the infestation occurs in cultivated fields. We are fortunate that this parasite has not yet been found in any cropping situation.

Some historical methods that have been used for control of Orobanche in other infested areas include: 1. planting of trap crops before commercial crops; 2. hand pulling; and 3. burying seeds deep into the soil by plowing. These practices have proven only marginally effective and not suited to our eradication effort for the Texas infestation (Wilhelm, Sagen, Hall, Rosenberg, Nichols, and Schlocker, 1965). More recent

practices for control of Orobanche have included methyl bromide fumigation (Wilhelm, Benson, and Sagen, 1958). This practice is very costly as well as having limitations in its use. This option could be employed later if the situation warrants.

In 1982, we conducted cursory testing of numerous herbicides for preemergence and postemergence control of Orobanche in the Texas site. Of the herbicides tested glyphosate, MSMA, cacodylic acid, and Cobra® were efficacious treatments in this non-crop situation. This research indicated that under the circumstances, glyphosate was found to be the most efficacious treatment for the control of emerged O. ramosa. We chose to use a treatment rate which is toxic but has sub-lethal effect on non-target species. Work by Schmitt, et.al. indicates Orobanche control by the translocation of small doses of glyphosate to developing underground roots of Orobanche (Schmitt, Schluter, and Boorsma, 1979). We presume some translocation of glyphosate for our control. Fortunately, for us, we need not be as concerned with damage to the host as one would in the situation where the parasite is attacking crops.

The successful use of ethylene gas in controlling Striga in the United States led to investigating its potential for Orobanche control (Eplee, 1975 and 1981). Encouraging results on the germination of O. ramosa by ethylene has been reported by the University of California. They (Chun, Wilhelm, and Sagen, 1979) reported that ethylene demonstrated the ability to reduce seed populations in the soil. Our preliminary studies indicate no significant stimulation with ethylene. We plan to conduct research on this subject through a cooperative program with Texas A&M University.

The program to eradicate this infestation of O. ramosa was begun this year. Our goal is to eradicate this infestation in 5 years. The program will include sufficient surveys to identify all sites of infestation. We will enforce a strict regulatory program to insure that the infestation does not spread to other areas. The population of Orobanche will be controlled using effective herbicides or other means to prevent seed production. If necessary, spot flaming will be used to destroy any escape plants that produce seed.

We are presently using a trap crop of flax to accelerate the demise of the population of seeds in the soil. The seed population in the soil will be determined and monitored by using a soil sampling procedure. This sampling process will permit us to measure progress and determine when eradication has been achieved.

We plan to conduct an ongoing research effort to insure the success of this program. We believe that in this case, containment and eradication is the best control measure. We are confident that we can eradicate this small infestation of O. ramosa from the U.S.A. before it has the opportunity to spread to other areas.

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THE DISTRIBUTION OF RHINANTHUS ANGUSTIFOLIUS IN RELATION TO HOST PLANT SPECIES

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Abstract. In grasslands with different soil fertility the frequency and size of Rhinanthus angustifolius differs. It was found that hosts from the most fertile soils tend to give a higher Rh. angustifolius production than those from less fertile soils. An exception are the Leguminosae. Host quality seems to be related to the nitrogen and phosphorus content of the host shoot, but host vitality influences the production of Rh. angustifolius as well. The hosts on which Rh. angustifolius performs best grow on the most fertile soils, but the highest population density is in grasslands with lower soil fertility and less favourable hosts. This means that the performance of individual plants can be explained in terms of hosts, but population numbers are subject to other restrictions.

INTRODUCTION

Addition of major nutrients can lead to marked changes in the composition of grasslands (Sonneveld et al., 1950; Vries and Kruijne, 1960; Thurston et al., 1976). Increase of nutrients causes a gradual elimination of slow growing species in favour of a few highly competitive fast growing species which are restricted to such sites.

In the brook valleys of the Drentsche Aa (Northern Netherlands) these different grassland types occur on peaty soils in close proximity (Grootjans, 1980). Rhinanthus angustifolius C.C. Gmelin occurs in those mainly mown for hay midsummer, with a second mowing or grazing later in the year. The vegetation types range from the very productive, species poor Poo-Lolletum with high soil fertility, to the less productive, species rich Lolio-Cynosuretum, Calthion and Juncetum-acutiflori grasslands with low soil fertility. The vegetation structure and microclimate differ with the species composition (de Hullu, 1984, in prep.). The frequency and the size of Rh. angustifolius vary considerably in the different grassland types.

In the Poo-Lolletum grasslands Rh. angustifolius occurs with a density of 30 - 120 m^{-2} and with very big plants with up to 150 capsules per plant. In the Lolio-Cynosuretum/Calthion grasslands Rh. angustifolius occurs with a density of 300 - 500 plants m^{-2}, the plants are much smaller, with up to 40 capsules per plant. In the Juncetum-acutiflori grassland the density is 30-120 m^{-2} and the plants are also rather small, with up to 50 capsules per plant.

This paper aims to analyse if the species composition of a grassland can influence the frequency and production per surface area of Rh. angustifolius, and tries to describe host quality in terms of nitrogen, phosphorus and potassium.

METHODS AND MATERIALS

Field situation

On the most fertile soils (N-indicator value 5-7) the Poo-Lolietum association is dominated by Lolium perenne, Agrostis stolonifera, Alopecurus geniculatus, Poa pratensis, Bromus hordeaceus, Ranunculus repens, Festuca pratense and Trifolium repens. The average capsule production of Rh. angustifolius in this field is 61.3/plant (95% confidence interval ± 124.4). On the soils of intermediate fertility (N-indicator value 4-5) the Lolio-Cynosuretum association is dominated by Anthoxanthum odoratum, Cynosurus cristatus, Holcus lanatus, Festuca rubra, Deschampsia cespitosa, Equisetum fluviatile, Lychnis flos-cuculi, Lotus uliginosus, Trifolium repens and Ranunculus acris. The average capsule production of Rh. angustifolius in this field is 7.1/plant (95% confidence interval ± 3.3.). On the least fertile soils (N-indicator value 3-5) the Lolio-Cynosuretum / Juncetum acutiflori association is dominated by Juncus acutiflorus, Anthoxanthum odoratum, Holcus lanatus, Carex nigra, Lotus uliginosus and Equisetum palustre. The average capsule production of Rh. angustifolius in this field is 14.0/plant (95% confidence interval ± 16.3).

EXPERIMENT I

This experiment aimed to analyse the quality of a number of hosts in these grasslands. The 18 host species were chosen because they had a high cover percentage in the vegetation, or a high (6-7), medium (4-5) or low (2-3) indicator value for nitrogen (Ellenberg, 1974). Three legumes were included, because species of this family are often considered good hosts for hemi-parasites (ter Borg and Bastiaans, 1973).

The species are divided into three groups.

- Group I (characteristic of nutrient rich soils): Bromus hordeaceus, Trifolium repens, Alopecurus geniculatus, Carex hirta, Lolium perenne, Alopecurus pratensis, Festuca pratensis.
- Group II (less nutrient rich): Lychnis flos-cuculi, Deschampsia cespitosa, Anthoxanthum odoratum, Lotus uliginosus, Festuca rubra, Cynosurus cristatus.
- Group III (the least nutrient rich): Festuca ovina, Trifolium dubium, Carex hostiana, Carex nigra and Danthonia decumbens.

The hosts were grown from seed except the Carex species, which were grown from tillers. All were grown in the greenhouse (August) on a nutrient solution. (10 ppm NH_4^+ - N, 173 ppm K^+, 115 ppm Ca^{2+}, 31 ppm Mg^{2+}, 102 ppm NO_3^- -N, 23 ppm $H_2PO_4^-$ -P, 84 ppm SO_4^{2-} - S, pH 6). The plants were grown in 3.5L reservoirs and continuously aerated, covered with a plastic plate in each of which 9 perforated (upper ø 6.5 cm) plastic pots were inserted. These small pots were filled with rock-wool and planted with young host plants. After three weeks germinated seeds of Rh. angustifolius were planted. The nutrient solution was replaced each week. At harvest the number of capsules of Rh. angustifolius was counted and the above ground dry matter of host and parasite weighed.

Results

The dry matter production of Rh. angustifolius strongly depends on the host species (Fig. 1). Production is highest with Bromus hordeaceus and Trifolium dubium, lowest with Carex hostiana and Danthonia decumbens.

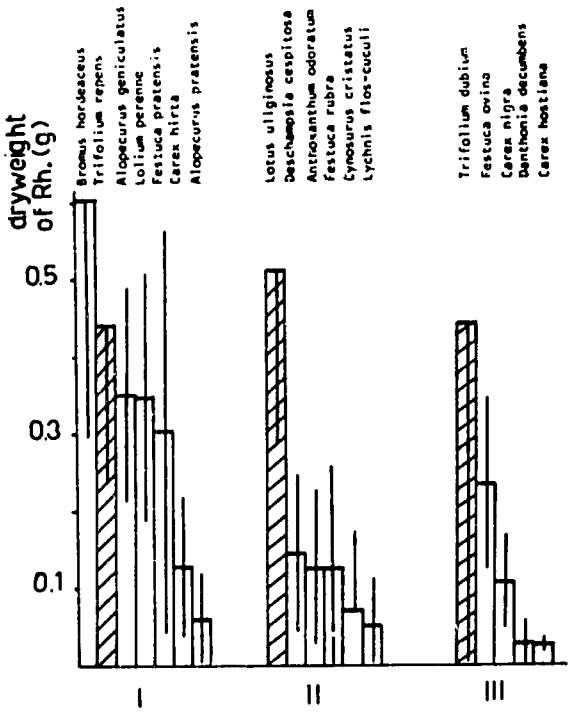


Fig. 1.
The dry weight production of Rh. angustifolius (mg), when grown with different hosts after 11 weeks. The hosts are selected from very fertile, intermediate and less fertile grasslands. Respectively I, II and III. 95% confidence interval is indicated.

The differences are considerable. Overall the hosts from the most fertile grasslands give the highest production of Rh. angustifolius. Between species within one group considerable differences do occur, especially in groups II and III. Rh. angustifolius with Lotus uliginosus and Trifolium dubium gives a very high production, while Carex hostiana and Danthonia decumbens result in a very low production.

There is a linear correlation of capsule production with dry weight at R = 0.76, and thus provides a measure of plant productivity. In the field also capsule production of Rh. angustifolius is higher in Poo-Lolietum than in Lolio-Cynosuretum.

EXPERIMENT II

An experiment was performed to analyse if host quality could be related to the nitrogen, phosphorus and potassium content of the host shoots. The shoot was chosen, because this is probably a measure for physiological activity of the ions. Most species in this experiment are the same as in Experiment I, and they were chosen with the same criteria.
- Group I: Lolium perenne, Alopecurus geniculatus, Trifolium repens, Carex hirta.
- Group II: Anthoxanthum odoratum, Festuca rubra, Lychnis flos-cuculi, Lotus uliginosus.
- Group III: Carex nigra, Carex hostiana, Danthonia decumbens and Festuca ovina, Trifolium dubium.

These species were planted in pots (upper Ø 12 cm) with 1 part fertilized peat potting soil and 2 parts coarse sand; after 3 weeks germinated Rh. angustifolius seeds were planted. The pots were watered daily with demineralized water. The shoots were harvested after 11 weeks and weighed oven-dry. Hosts and parasite were pooled in two batches of 5 pots to be analyzed for total nitrogen concentration by the Kjeldahl method (Wieniger, 1936), for total phosphorus concentration by a colorimetric molybdate method (Murphey and Riley, 1962) and for potassium with flame absorption photometry.

Results

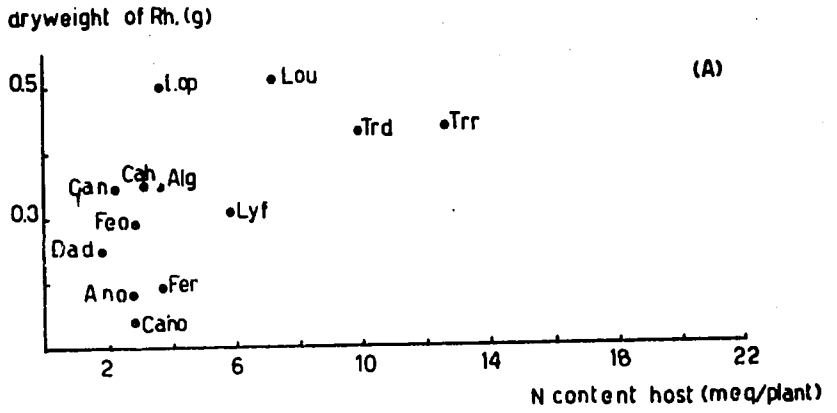
Rh. angustifolius tends to have a higher dry weight production with an increased nitrogen (Fig. 2A) and phosphorus (Fig. 2B) content of the host shoot. Leguminosae clearly form a special case with high nitrogen content and generally higher phosphorus content. The dry weight production of Rh. angustifolius shows no correlation with the potassium content of the host shoot (Fig. 2C).

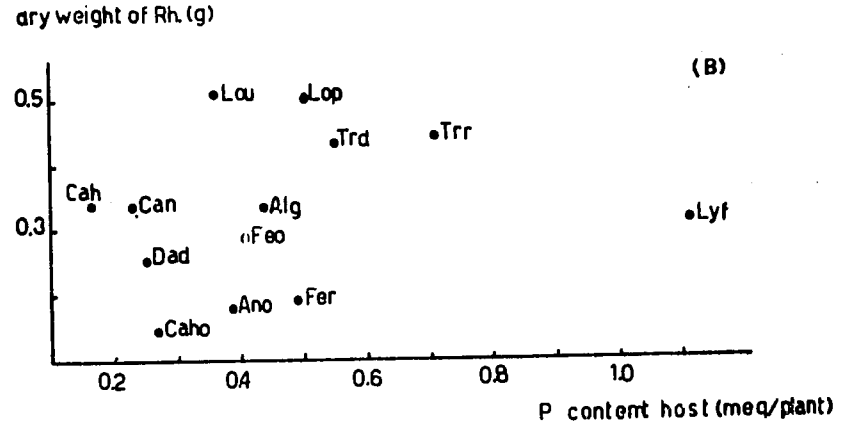
EXPERIMENT III

The production of Rh. angustifolius seems to be correlated with the nitrogen and phosphorus content of the host shoot (Fig. 2A, B). An experiment was designed to test this in a field situation. In a Lolio-Cynosuretum vegetation with a relatively nutrient poor soil, 4 random plots of 80 x 80 cm were fertilized in spring with nitrate (200 kg/ha), phosphate (100 kg/ha) and potassium (100 kg/ha). This should result in a higher nutrient content of the plants (Fitter and Hay, 1982). In July plants were harvested from these plots and from 4 control plots. By than the composition of the fertilized plots was still similar to that of the control plots.

Results

The capsule production per plant increased significantly from 11 ±4.5 per plant to 27 ± 28.5 per plant (± s.d.) when the field was fertilized. However, the standard deviation widened markedly as well. This means that not all Rh. angustifolius plants show the same reaction.





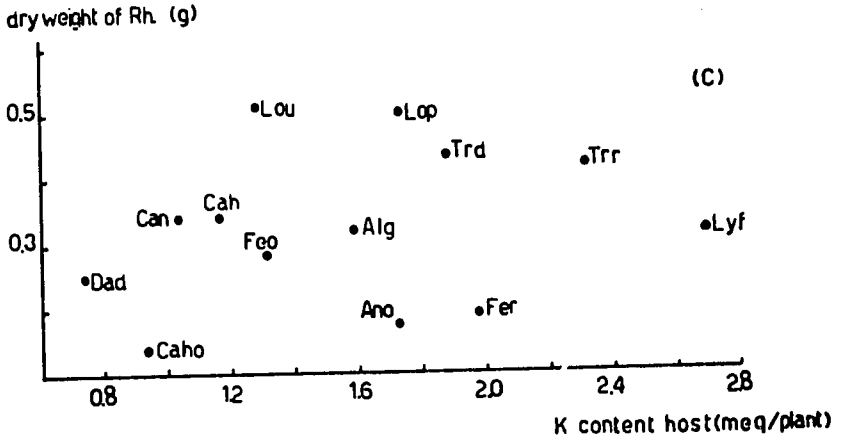


Fig. 2A, B, C.
The relation between the nitrogen (A), phosphorus (B) and potassium (C) content of the host (meq./plant) and the dry weight production (g) of Rh. angustifolius.
Al.g = Alopecurus geniculatus, An.o = Anthoxanthum odoratum, Ca.h = Carex hirta, Ca.ho = Carex hostiana, Ca.n = Carex nigra, Da.d = Danthonia decumbens, Fe.o = Festuca ovina, Fe.r = Festuca rubra, Lo.u = Lotus uliginosus, Ly.f = Lychnis flos-cuculi, Tr.d = Trifolium dubium, Tr.r = Trifolium repens.

Fertilizer application changes the vegetation structure considerably. Most plants produce more biomass, and therefore the light regime and microclimate changes. Some Rh. angustifolius plants seem unable to adapt.

EXPERIMENT IV

The fertilizer experiment in the field showed that the production of Rh. angustifolius could be different despite an unchanged species composition.

To test the influence of the vitality of the host plant on the production of Rh. angustifolius an experiment was designed with Lolium perenne. L. perenne seedlings were grown in a pot (Ø 12 cm) with 1 part fertilized peat and 4 parts perlite. After 1 month 4 germinated Rh. angustifolius seeds were planted, and the pots were treated variously in order to get a different nutrient regime to affect the vitality.

The applied nutrient solutions were Hoagland & Spencer 0.5 and 1.0 normal strength, 0.5 and 1.0 normal strength without nitrogen, 0.5 and 1.0 normal strength without phosphate, and 0.5 and 1.0 normal strength without potassium, and demineralized water throughout.

Every nutrient sere had 10 replicates. After 10 weeks Rh.angustifolius and L. perenne were harvested and shoot dry matter production was measured.

Results

In the 1.0 normal strength Hoagland series complete, the production of L. perenne is higher, but that of Rh. angustifolius lower than in the 0.5 normal strength Hoagland series (Fig. 3) except in the nitrogen deficient solution, where the production of both Rh. angustifolius and L. perenne is very low. Production was also low with demineralized water.

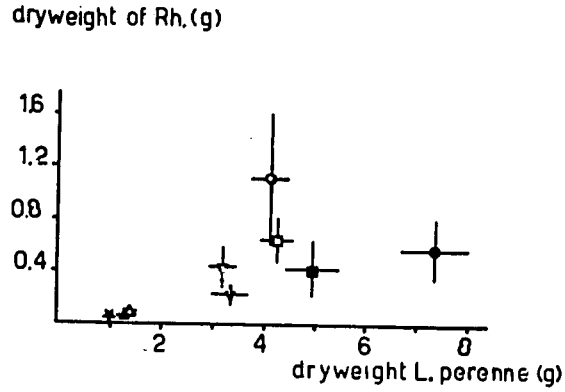


Fig. 3. The relation between the dry weight production of Lolium perenne (g) and Rh. angustifolius (g) when grown under different nutrient regimes. 95% confidence intervals are indicated. ○ open symbols indicate grown on 0.5 normal strength Hoagland, ● solid symbols indicate grown in 1.0 normal strength Hoagland, ○ complete Hoagland solution, □ P-deficient Hoagland solution, ▽ K-deficient Hoagland solution, △ N-deficient Hoagland solution, ★ demineralized water.

The experiment shows that not only the host species, but also the nutrient and growth condition of the host influence the production of Rh. angustifolius . Very vigorously growing hosts are less sensitive to an attack by Rh. angustifolius than less vigorously growing hosts, in which is no shortage of nutrients. This deminished production of L. perenne is not likely to be due the effect of the parasite as is known that under these circumstances Rhinanthus does not affect the host (Weber, 1976). However when there is a shortage of the nutrients K, P, and especially N, the dry weight production of Rh. angustifolius deminishes considerably.

DISCUSSION

Host quality in relation to size of Rhinanthus angustifolius

It is generally observed (Tsivion, 1978; Klaren, 1975 ; Klaren and Jansen, 1978; Lamont and Southall, 1982) that after attachment both growth rate and nutrient status increase in hemi-parasites. Weber (1976) shows that with some host species no haustorial formation takes place. But when haustoria formation does take place not all hosts can be exploited to the same extent. Host specificity is unknown for many hemi-parasites, most are able to attack a relatively wide range of species (Yeo, 1964; ter Borg,1972; Parker and Reid, 1979; Snogerup, 1982). Differences in host quality are known for Euphrasia (Yeo, 1964), Odontites (Snogerup, 1982), Orthocarpus (Atsatt and Strong, 1970), Striga (Wilson Jones, 1955) and Rhinanthus (ter Borg, 1972; ter Borg and Bastiaans, 1973). In the group of good hosts for Rh. angustifolius (ter Borg, 1972; Campion-Bourget, 1982) considerable differences in quality are exhibited as experiment I shows. However, these results must be treated with care, because host quality is not a constant factor. Genetic adaptation is suggested both for host (Snogerup, 1982) and parasite (Wilson Jones, 1955). Experiment IV shows that host vitality can be an important factor.

Most of the above mentioned authors pay special attention to the Leguminosae. In general they are considered to be very good hosts, but there are a number of exceptions (Snogerup, 1982). Ter Borg and Bastiaans (1973) show that Trifolium repens performs very well for Rh. angustifolius and also Trifolium dubium, Trifolium repens, Lotus uliginosus are very well-suited (Experiment I).

The best non-Leguminosae hosts for Rh. angustifolius occur in group I (Experiment I). These are species which grow on fertile soils. This phenomenon and the fact that Leguminosae fix nitrogen and the uptake of nitrogen can stimulate the uptake of phosphate directly support the suposition that increased nitrogen and phosphorus content results in increased Rh. angustifolius production (Figs 2A,B). However, this is not a simple relationship; obviously there are other factors in the host that affect the quality (Figs. 2A, B).

When the host grows vigorously (is very competitive) the production of Rh. angustifolius decreases again (Fig. 3). Ter Borg (1972) stated that fast growing annuals are not good hosts for Rhinanthus due to their high competitive ability, and Atsatt and Strong (1970) stated that the best hosts were those susceptible to over-parasiting, which means that they have a low competitive ability. The competitive ability of host seems to be an

important part of its quality.

The observed differences in the measured parameters within the experiments are big (e.g. Fig. 1). This variation is mentioned in nearly all work with hemi-parasites. Wilson Jones (1955) and Atsatt and Strong (1970) suggest that the basis for this variation is genetically based. Selection is not likely to be an explanation for these experiments. Seeds were selected from the species rich haylands and the chance that a Rh. angustifolius plant parasitizes the same host species in successive years is small.

Host quality in relation with population density of Rh. angustifolius

In the species poor,nutrient rich grasslands, the density of Rh. angustifolius is fairly low (30-120 m^{-2}) and the seed production per plant very high. Some species from these grasslands (group I) show a very high production of Rh. angustifolius and therefore a high seed production. This means that the growth conditions for Rh. angustifolius must be very favourable. However, the highest Rh. angustifolius densities (300 m^{-2}) occur in the species-rich less nutrient-rich grasslands, in which the species are less good hosts (group II) and give a moderate amount of seeds per plant. This results in a comparable seed production m-2 in these grassland types (de Hullu, in prep.).

The nutritional condition of the soil is less good than in group I. This means presumably that other factors restrict the density of Rh. angustifolius in the most fertile grasslands. The factor that limits the density is obviously not the seed production, because it is not likely that under natural conditions the hosts are so vigorous (Experiment IV) that this limits the production of Rh. angustifolius. The limiting factor for establishment and development of hemi-parasites is possibly the structure (de Hullu, 1984) or light and microclimate (Yeo, 1964; Heinricher, 1910; de Hullu, in prep.) and partly probably the nutrient conditions of the soil. In the less fertile grasslands the structure is more open and heterogeneous, because of the species richness. In these grasslands the structure is more suitable for Rh. angustifolius growth and development. In the Lolio Cynosuretum / Juncetum acutiflori vegetation the soil is nutrient-poor, the structure is rather open. In this vegetation type a combination of the nutrient conditions of the soil and host quality is probably limiting, and results in a low density of Rh. angustifolius.

Rh. angustifolius shows here that the maximum biomass production per plant does not reaches its maximum density per unit area in the same grassland type. This agrees with the conclusions of Mizianty (1975), who also finds the highest population density of Rh. angustifolius in the species-rich vegetation types and not in the very productive grasslands where the few Rhinanthus plants are biggest.

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SOME TAXONOMIC PROBLEMS IN THE GENUS STRIGA WITH PARTICULAR REFERENCE TO AFRICA

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Abstract. Four species are briefly discussed relative to variation and possible relationships to other species. Striga gesnerioides (Willd.) Vatke has four well defined, host specific races — one each on Euphorbia, Convolvulaceae, cowpea, and tobacco. S. asiatica (L.) Kuntze and S. elegans Benth. differ mainly in the size of the corolla and some specimens appear to be intermediate. S. euphrasioides (Vahl) Benth. is very similar to S. masuria Benth., a species of East Asia. S. passargei Engl. is a variable, ill defined species which shows some similarities to the Indian S. densiflora Benth.

INTRODUCTION

The genus Striga Lourierio comprises perhaps 30 species of which about 23 occur in Africa. Striga species are characterized by a preference for open grasslands in semi-arid regions. All species that have been examined are parasites and it is safe to assume that this behavior is found throughout the genus. Morphologically, the genus is separated from others in the subfamily Rhinanthoideae by the unilocular anthers and bilabiate corollas with a pronounced bend in the corolla tube. Striga is related to Buchnera which has an almost radially symmetrical corolla and lacks a bend in the tube.

Despite the great economic impact of several species on some of the most important food crops in Africa, little attention has been paid to the systematics of the genus as a whole, especially to the relationship among species. For this reason I am discussing four species which appear to have closely related counterparts or, in the case of S. gesnerioides, to have well developed strains.

METHODS AND MATERIALS

Field work in Africa was conducted in Upper Volta, Nigeria and Senegal during the past ten years with the majority in Sudan. For herbarium studies I have examined material, including type specimens, at Zurich (Z), Paris (P), Kew (K), British Museum (BM), Edinburgh (E) as well as the smaller herbaria of the Weed Research Organization (WRO), University of Khartoum (KHU), Gezira Research Station (WM), Forestry Research Centre, Khartoum (KHF), and Old Dominion University (ODU).

RESULTS AND DISCUSSION

Infrageneric classification

To date, the only infrageneric classification is that of Wettstein (1893) who erected two sections based on the number of ribs in the calyx. Section Pentapleureae Wettstein has five ribs and each rib terminates a calyx lobe. In Section Polypleureae Wettstein, the calyces have ten or more ribs, the alternate ribs terminating at the sinus of the calyx tube or sometimes, in species with 15 ribs, with three ribs terminating a lobe.

How much these sections reflect true relationships within the genus is unclear. The number of ribs may vary even on the same plant. For example, it is not unusual to find a calyx of S. asiatica (Section Polypleureae) with 11 ribs on a inflorescence branch where most have ten. However, any species of Striga clearly fits unequivocally into one or the other sections.

Species groups

1. Striga gesnerioides (Willd.) Vatke

This is a very distinctive species characterized by scale-like leaves, pubescent corolla, and a large primary haustorium. It is found throughout Africa where four distinct races or morphotypes may be found. These are discussed below. Unlike Saldanha (1963) I do not ascribe any formal taxonomic status to these races, at least until further work is carried out.

a. Euphorbia strain. This is found in Northwest Africa, the Red Sea Hills of Egypt, Sudan and Yemen (and Ethiopia?) and is also reported from India. It parasitizes succulent species of Euphorbia and is distinguished by the cauline branching, dark purple flowers, dark red stems, and large (up to 3.5 cm wide) single primary haustorium. The haustorium often perennates, making this the only perennial strain in the species complex.

b. Convolvulaceae strain. This is widespread throughout the Sahel. Frequent hosts include species of Ipomoea, Meremmia, and Jacquemontial. This strain usually has pinkish blue flowers and may be either sparsely or profusely branched. Most frequently it is found on Convolvulaceae but also possesses the ability to attack legumes. For example, in the South Kordofan region of Sudan, the usual host is Meremmia emarginata or Jacquemontia tanmifolia and only very rarely Indigofera nummulariifolia (a legume). This host selection phenomenon was reported earlier by Musselman and Parker (1981) working with a strain of S. gesnerioides introduced into the United States and should be considered when introducing legume crops in areas where this strain is present.

c. Cowpea strain. This is the best known of all the strains but is not always easily distinguished from the above. It has much branched stems and bluish flowers.

d. Tobacco strain. Known only from Zimbabwe and South Africa, this strain has unbranched stems and plicate petals.

Earlier work (Musselman and Parker, 1981) had shown strains b, c, and d to be host specific. The Convolvulaceae strain was also grown at the University of Khartoum on Meremmia emarginata but failed to grow on any other host including tobacco, cowpea, and Euphorbia. It has not yet been possible to germinate seed of the Euphorbia strain. All of these strains are autogamous.

2. Striga asiatica (L.) Kuntze

Striga asiatica is the most widely distributed Striga species in the world being found throughout much of Africa, Southwest Arabia, India, Burma, China, New Guinea, the Philippines, Indonesia, and perhaps Australia. Striga elegans Benth., on the other hand, is restricted to East and South Africa. The main distinction between the two is the size of the corolla, those of S. elegans are up to ca. 2 cm across, those of S. asiatica seldom more than 1 cm wide. As Hepper (personal communication) had noted, the flowers of S. elegans persist for more than one day while those of S. asiatica last only one day. The brilliant display of the inflorescence in S. elegans naturally suggests a role for a pollinator and it would not be surprising to find a group of insects that visit the flowers, perhaps similar to that of S. hermonthica which is pollinated by bee flies (Diptera) and moths and butterflies (Lepidoptera). Earlier work (Nickrent and Musselman 1979) has shown S. asiatica to be autogamous so it would be interesting to study the floral biology of S. elegans. Is S. asiatica an autogamous offshoot of S. elegans?

3. Striga euphrasioides Benth.

This is one of a group fo Striga species that has 15 calyx ribs. In S. euphrasioides the ribs are very prominent. S. euphrasioides is mainly Indian in distribution and is otherwise found only in a small area of East Africa. Striga masuria Benth., on the other hand, is found in western China, Indonesia, and New Guinea. In Asia the two species are geographically separated by a relatively small distance and are sympatric in Indonesia. The discovery of S. euphrasioides in the Dhofar region of Oman provides a "bridge" between the Indian and African populations. These similarities in morphology and distribution invite questions as to the relationship between the two species.

Bentham (1835) noted that the chief difference between S. euphrasioides and S. masuria was the much larger corolla of the latter. However, I have examined specimens of both species from throughout their range and find considerable variation in plant and corolla size. This does not seem surprising as S. euphrasioides is known to be capable of germinating without a host stimulant. It thus has the potential of being a faculative parasite. Such parasites are characterized by considerable variation in vigor including corolla size. Further work is needed to determine the source of variation within these two species and to elucidate the significance of their obvious similarities.

There remains considerable confusion over the correct name for the plant we are discussing as S. euphrasioides. Saldanha (1963) has shown that the type specimen of Buchnera euphrasioides Vahl is not the same plant as that generally known as S. euphrasioides and proposed the name S. augustifolia. I have examined photographs of the type and agree that the specimen of Vahl is not S. euphrasioides. However, there is still some confusion over the name which should be based on Vahl's specimen. Until this is resolved, I am retaining the name S. euphrasioides Benth. while acknowledging the problem of typification.

4. Striga passargei Engl.

Essentially African in distribution with a few collections from the Southwest Arabian penninsula, S. passargei is a variable and poorly circumscribed species. Like all Striga species, the size of the individual plants varies considerably but most striking is the variation in corolla color which may be yellow, cream, white, pink or white with a pink center. It is often confused with S. aspera which has, however, a more racemose inflorescence and short floral bracts while S. passargei has axillary flowers and very long bracts which greatly exceed the leaves. Most variable however, is the size of the corolla, especially the width of the limb. In some specimens, the limb measures 3 mm across while in others it is scarcely 1.5 mm. Rather than S. aspera, I find the Indian species S. densiflora Benth. difficult to separate from S. passargei. Apparently the chief difference is in the somewhat shorter floral bracts of S. densiflora.

Further work

Obviously, considerable effort is necessary to clarify relationships within the genus Striga. The information presented here is only a necessary first step of identifying some of the problems. Systematic studies in the genus would be facilitated by additional herbarium specimens from the range of the species. This is often difficult, however, as the phenology of Striga species is highly seasonal so that unless a special effort is made in collecting, the flowering season is easily missed. The obligate parasitism is only a small hindrance to the growth of the parasites as attempts to grow species on suitable hosts is generally successful. Such a procedure is necessary for the crosses which will be helpful in elucidating relationships.

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PHYSIOLOGICAL SPECIALIZATION OF STRIGA HERMONTHICA AND CROP SPECIFICITY

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Abstract Six samples of Striga hermonthica (Del.) Benth. from sorghum and eight from millet (Pennisetum americanum (L.) K. Schum) were tested against two susceptible cultivars each of sorghum and millet in 1981 and 1982 pot experiments to present evidence for crop specificity. The Striga seed samples were collected from latitudes ranging from 10°42'N to 13°29'N and thus represent a narrow geographical distribution of Striga hermonthica. The results indicate that sorghum cultivars were able to germinate and support Striga from both sorghum and millet hosts whereas millets could only support Striga from millet. However, in certain regions both the crops were attacked by both types of Striga. Extreme host specificity was not exhibited by the Striga samples included in the present investigation. They represent intermediate forms which were able to attack sorghum more than millet since sorghum is more extensively grown in the region under study. In areas where millet Striga samples were collected they attacked millet but also retained the capacity to attack sorghums. It is suggested that specialization is the outcome of the intensity with which a particular host crop is grown at the exclusion of the other thus creating a reproductive isolation between sorghum and millet Striga strains.

INTRODUCTION

Striga hermonthica (Del.) Benth. is wide-spread in the semi-arid zones of northern tropical Africa from 5°S to 20°N latitude. From East to West it is distributed across the width of Africa. It attacks several food crops like sorghum, millets, maize, upland rice, sugarcane, and also several wild grasses in fallow lands and field bunds. This particular species of Striga is cross pollinating and thus able to generate genetic variability for its pathogenicity. Inspite of genetic variability no morphological differences have been reported in this species even though different morphophytes exist in Striga asiatica (L.) Kuntze a self pollinating species (Ramaiah etal 1983). Different forms of Striga hermonthica have not become fixed and maintained because of continuous cross pollination and exchange of genes within the Striga population. However, several detailed investigations including laboratory and field experiments by Wilson Jones (1955) and Bebawi (1981) in the Sudan, by King and Zummo (1977) and Ramaiah (1983) in West Africa and by laboratory experiments of different samples from Africa (Parker and Reid 1979) have established that there are atleast two biotypes in Striga hermonthica, one specific to millet (Pennisetum americanum (L.) K. Schum) in the relatively drier and low rainfall zones where millet is the major cereal and another biotype specific to sorghum in areas where sorghum is the predominant crop grown. The crop specificity of these extreme forms is reported to be based on different germination stimulant requirements (Parker and Reid, 1979) in contrast to the report by Wilson Jones (1955) that specificity is due to

factors that operate after germination. Our own field observations and pot experiments in West Africa (ICRISAT 1980) confirmed that this physiological specialization is true when one considers the extreme Striga samples particularly those collected from millet host, those from Maradi in Niger and Bambey in Senegal which represent extreme drier zones where sorghum is almost absent. But there are regions is West Africa where both sorghum and millet act as hosts (Ramaiah, 1983). A preliminary report of the variability in pathogenicity in relation to host crops and geographical distribution revealed that this species varies from North to South but very little from East to West (Ramaiah, 1983). At Maradi, Niger and Bambey, Senegal which are North of latitude 13°N Pennisetum millet is the predominant host whereas in Kamboinse Upper Volta, Kobo, Ethiopia, Abu Naama and Wad Medani, Sudan, and Busia, Kenya sorghum is the principal host. Striga was found to be attacking both sorghum and millet crops in such locations as Farako-Bâ, Upper Volta and Mintimbougou, Mali where both the crops are grown. Host specificity has been previously reported (Wilson Jones, 1955., King and Zummo, 1977., Parker and Reid, 1979., and Bebawi, 1981). The present study, limited to latitudes ranging from 10°42'N in Upper Volta includes samples of Striga collected from sorghum and millet in the same latitude zone for detailed crop specificity studies.

MATERIALS AND METHODS

1981:

Seven Striga seed samples collected in different rainfall zones of Upper Volta from pure millet fields and three from pure sorghum fields were tested against two susceptible cultivars of sorghum (CK60B and IS9289) and two susceptible cultivars ofmillet (ICH241 and ICH7819) in a split plot design with four replications. The details are given in Table 1.

1982:

Eight Striga samples, four from sorghum and four from millet, were tested against two susceptible cultivars of sorghum (CK60B and BTx623) and two susceptible cultivars of millet (Ex-Bornu and ICH241) in a split plot design with six replications. The details are given in Table 1.

Striga seeds of the same source could not be used in both years because of the shortage of seeds. As seen from the table the samples used in both the years are from about the same latitude zones. In both years split plot design was used with the cultivars as main plots and Striga as sub plots. The experiments were carried out in 5 kg pots in the rainy seasons of each year. Soil, sand, and farmyard manure were used in the proportion of 50:25:25. by volume. Approximately 6,500 Striga seeds per pot were mixed in the top 5 cm soil before sowing the host crop seeds. Four to five seeds of host crop were sown in each pot at a depth of 3-4 cm and thinned to one plant later. Additional fertilizer was not used. Pots were irrigated as required.

Table 1

Details of Striga hermonthica seed samples used in the experiments

Striga code	Location (Upper Volta)	Latitude (approximate)	Host crop	Year of collection	Year of extpt.
STS-25	Banfora	10°42'N	Sorghum	1981	1982
STS- 7	Zandkom	13°08'N	"	1979	1982, 1981
STS-17	Tangaye	12°10'N	"	1979	1982
STS-13	Djibasso	13°07'N	"	1979	1982
STS-14	Fada N'Gourma	12°02'N	"	1979	1981
STS-16	Kamboinsé	12°28'N	"	1979	1981
STP-21	Banfora	10°42'N	Millet	1981	1982
STP-10	Korsimoro	12°49'N	"	1979	1982
STP-15	Tangaye	12°10'N	"	1980	1982
STP-12	Djibasso	13°07'N	"	1979	1982
STP- 4	Zitango	11°08'N	"	1979	1981
STP-11	Roumassi	13°06'N	"	1979	1981
STP- 8	Koupela	12°11'N	"	1979	1981
STP- 1	Ouahigouya	13°29'N	"	1979	1981

Counts of emerged Striga were made from the date of first emergence at intervals of two weeks. The maximum emergence was 64 and 70 days after sowing in 1981 and 1982 respectively. The data were transformed using log (X+1.1) transformation and analysed. In each trial analysis was carried out separately for millet and sorghum Striga.

RESULTS

1981 Striga from millet:

Results presented in Table 2 indicate no significant differences among the cultivated varieties though sorghum cultivars supported more Striga than millet cultivars. The differences among Striga samples and the interaction between Striga and cultivars were highly significant. The emergence of STP-4 and STP-8 Striga samples was highest and lowest respectively. STP-11 and STP-1 were intermediate and not significantly different from each other. STP-4 and STP-1 did not interact significantly with the host crops whereas STP-11 and STP-8 interacted significantly. STP-8 attacked sorghum more than millet whereas STP-11 did not show any specificity to the crops.

1981 Striga from sorghum:

The results presented in Table 3 show significant differences between cultivars, between Striga samples and for the interaction

Table 2

Effect of crop cultivars on emergence of S. hermonthica originating from millet host (1981): Striga emergence per pot 64 days after sowing (log X+1.1)

Cultivars		Striga[c]				cultivar mean[a]
		STP-4	STP-11	STP-8	STP-1	
CK60B	(Sorghum)	1.46	1.23	1.02	0.89	1.151
IS 9289	(Sorghum)	1.08	0.79	0.54	0.99	0.850
ICH241	(Millet)	1.06	0.78	0.11	1.02	0.742
ICMV7819	(Millet)	1.16	1.00	0.30	0.78	0.809
Striga mean[b]		1.193	0.950	0.493	0.920	

a = LSD (5%) for cultivars = 0.342
b = LSD (5%) for Striga = 0.142
c = LSD (5%) for Striga at same cultivar level = 0.283
LSD (5%) for cultivars at same Striga level = 0.420

between Striga and cultivars. Sorghum supported emergence of these Striga samples significantly more than millet, but the difference was small in the case of STS-7. Sorghum cultivars did not interact significantly with the Striga samples in contrast to millet cultivars. ICH241 interacted differently with all the Striga samples whereas ICMV 7819 supported significantly fewer plants of STS-16.

Table 3

Effect of crop cultivars on emergence of S. hermonthica originating from sorghum hosts: Striga emergence per pot 64 days after sowing (log X+1.1)

Cultivars	Striga[c]			cultivars mean[a]
	STS-7	STS-14	STS-16	
CK60B	1.25	1.31	1.25	1.270
IS 9289	1.15	1.18	1.23	1.186
ICH241	0.97	0.42	0.15	0.513
ICMV 7819	0.71	0.87	0.48	0.688
Striga mean[b]	1.019	0.946	0.778	

a = LSD (5%) for cultivars = 0.149
b = LSD (5%) for Striga = 0.257
c = LSD (5%) for Striga at same cultivar level = 0.257
LSD (5%) for cultivars at same Striga level=0.257

1982 Striga from millet:

The results presented in Table 4 indicate no significant differences among the cultivars whereas Striga samples exhibited significant differences. There was also a highly significant interaction. STP-21 gave highest germination and STP-10 the lowest. STP-15 and STP-12 were intermediate. STP-21 and STP-12 did not interact significantly with host cultivars whereas STP-10 and STP-15 did interact significantly. STP-10 was germinated significantly more by millet compared to sorghum where STP-15 was germinated significantly more by sorghum than by the millet.

Table 4

Effect of crop cultivars on emergence of S. hermonthica originating from millet (1982): Striga emergence per pot 70 days after sowing (log X+1.1)

Cultivars	Striga [c]				cultivars mean[a]
	STP-21	STP-10	STP-15	STP-12	
CK60B	1.56	0.42	1.32	1.31	1.150
BTx623	1.56	0.04	1.45	1.02	1.029
Ex-Bornu	1.45	1.55	0.99	1.20	1.285
ICH241	1.56	1.12	0.97	1.03	1.172
Striga mean[b]	1.534	0.784	1.180	1.140	

a = LSD (5%) cultivars = 0.281
b = LSD (5%) Striga = 0.185
c = LSD (5%) for Strigas at same cultivar level = 0.369
LSD (5%) for cultivars at same Striga level= 0.426

1982 Striga from sorghum:

The results presented in Table 5 reveal significant differences among cultivars and among Striga samples. The interaction between cultivars and Striga was also significant. Sorghum cultivars supported significantly more Striga than millet. STS-25 and STS-13 did not interact significantly with the host cultivars while both STS-7 and STS-17 emerged out of the soil significantly more with sorghum cultivars than with millet cultivars.

DISCUSSION

In the present investigation out of eight Striga samples from millet studied in 1981 and 1982, five samples STP-4, STP-11, STP-1, STP-21, and STP-12 have not shown any crop specificity whereas

Table 5

Effect of crop cultivars on emergence of S. hermonthica originating from sorghum (1982): Striga emergence per pot 70 days after sowing (log X+1.1)

Cultivars	Striga[c]				Cultivar mean[a]
	STS-25	STS-7	STS-17	STS-13	
CK60B	1.74	1.40	1.67	1.10	1.478
BTx623	1.52	0.65	1.72	1.10	1.247
Ex-Bornu	1.39	0.04	0.39	0.77	0.647
ICH241	1.29	0.25	0.45	0.87	0.717
Striga mean[b]	1.485	0.59[illegible]	1.056	0.961	

a = LSD (5%) for cultivars = 0.234
b = LSD (5%) for Striga = 0.253
c = LSD (5%) for Striga at same cultivar level = 0.518
LSD (5%) for cultivars at same Striga level = 0.507

STP-10 (from Korsimoro) has exhibited partial specificity to millet while STP-15 (from Tangaye) and STP-8 (from Koupela) attacked the sorghum cultivars more than the millet.

On the contrary a majority of Striga samples from sorghum have shown specificity to sorghum. Among the six Striga samples tested in both the years four Striga samples viz., STS-7, STS-14, STS-16, and STS-17 have exhibited specificity to sorghum whereas STS-25 and STS-13 did not show any specificity but attacked both the crops. STS-7 was tested in both the years and maintained its crop specificity. Two sets of Striga samples are of special interest. STP-12 and STS-13 (from millet and sorghum respectively from Djibasso) and STP-21 and STS-25 (from millet and sorghum respectively from Banfora). In each case the pairs of samples attacked both crops without showing any crop specificity. This is in contrast to Wilson Jones (1955) findings that S. hermonthica from sorghum and millet Kordofan, Sudan attacked more millet cultivars than sorghum cultivars thus suggesting crop specificity. However, other Striga samples from sorghum (STS-14) and millet (STS-8) collected from the same latitude zone (around 12°N) attacked sorghum cultivars significantly more than the millet cultivars indicating that this is principally a sorghum strain. The sorghum cultivars tested were able to germinate and support most of the Striga samples tested irrespective of the host crop from which they were harvested within the latitude zone that is under investigation whereas millet cultivars could only germinate Striga from millet and two of the samples from sorghum. Sorghum root exudate may thus include the stimulant compounds that could stimulate both the groups of Striga whereas millet root exudate has only the stimulant compound that is specific to millet Striga. Debawi (1981 personal communication) reported that sorghum root

exudate germinated more of sorghum Striga than millet Striga in the Sudan but with increase in temperature it also germinated more of millet Striga. At higher temperatures host specificity tended to disappear. Parker and Reid (1979) using two samples each of sorghum and millet Striga against sorghum and millet root exudates demonstrated clear crop specificity based on stimulant compounds in the root exudates. These results agree with those of Ramaiah (1983) where the Striga strains show extreme specialization. The Striga samples studied in this present investigation perhaps represent intermediate forms of specialization as they are found only within a limited geographical range in which both sorghum and millet are grown.

Inspite of statistically significant differences, millet cultivars were able to support emergence of a proportion of sorghum Striga in our own experiments as well in other published reports. Given enough time in evolutionary process sorghum Striga could adapt to millet cultivars. This is proved by two Striga samples, STS-25 and STS-13, where intensity of attack on millet cultivars was comparable to that on sorghum cultivars. Similarly one could also hypothesize that Striga could develop extreme crop specificity in regions where only one host crop is grown. Examples are millet Striga from Maradi, Niger, and from Bambey, Senegal (King and Zummo, 1977; Parker and Reid, 1979; Ramaiah, 1983) and sorghum Striga from Kamboinse in Upper Volta (Ramaiah, 1983).

It is hypothesized that the S. hermonthica seed populations are genetically heterogeneous and respond to stimulant substances produced by roots of both sorghum and millet crops. During the course of evolution depending upon the host crop that is predominantly grown at the exclusion of the other, thus creating reproductive isolation between sorghum and millet Striga, the proportion of Striga seeds that respond to that particular crop root exudate buildsup whereas the other form remains in a smaller proportion but does not disappear completely because of long viability. The genetic basis of this crop specificity needs to be investigated.

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GLUTAMINE SYNTHETASE ISOENZYMES OF ANGIOSPERM PARASITES

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Abstract. Elution profiles of multiple forms of glutamine synthetase from leaf or shoot tissue of angiosperm stem and root parasites were obtained from ion-exchange chromatography and identified as cytosolic (GS_1) or chloroplastic (GS_2) enzymes using immunological techniques. Both GS_1 and GS_2 were present in the chlorophyllous leaf tissue of the hemi-parasite, Melapyrum arvense L. (ratio of GS_1 to GS_2 = 30:70), the obligate stem parasite, Arceuthobium oxycedri (D.C.) M. Bieb. (ratio of GS_1 to GS_2 = 90:10) and the obligate root parasites, Striga hermonthica (Del.) Benth. and S. gesnerioides (Willd.) Vatke. (ratio of GS_1 to GS_2 = 90:10). In contrast, the achlorophyllous stem (Cuscuta australis L.) and root (Orobanche minor Smith; O. cernua Loefl.; O. hederae Duby; O. ramosa L.; Lathraea clandestina L. and L. squamaria L.) parasites and non-photosynthetic tissue of S. gesnerioides possessed only one form of glutamine synthetase, identified immunologically as the cytosolic enzyme. The possible physiological role(s) in ammonia assimilation of each of the GS isoforms is discussed in relation to the parasitic habit.

INTRODUCTION

Glutamine synthetase (GS) catalyses the following reaction: L-glutamate + NH_4^+ + ATP $\xrightarrow{Mg^{2+}}$ L-glutamine + ADP and Pi, and is considered to be the major enzyme involved in ammonia assimilation in higher plants (Miflin and Lea, 1976). Moreover, glutamine synthetase has been implicated to function in the reassimilation of ammonia released during photorespiration and the catabolism of nitrogenous transport and storage compounds (Keys et al., 1978; Miflin et al., 1980). These multiple physiological functions are carried out by different isoforms of glutamine synthetase which can occur in various plant organs. In photosynthetically active tissue of barley (Hordeum vulgare L.) (Mann et al., 1979), pea (Pisum sativum L.) (Hirel and Gadal, 1981) and sorghum (Sorghum vulgare L.) (Hirel and Gadal, 1982), two forms of glutamine synthetase have been identified by ion-exchange chromatography. Subcellular localization (Mann et al., 1979) and immunocytochemical (Hirel et al., 1982) studies have shown one isoform to be present in the cytosol (GS_1) and the other isoform localized in the chloroplast (GS_2). The two leaf isoforms display different kinetic, regulatory and physiochemical properties (Mann et al., 1980; Ahmad et al., 1982; McNally et al., 1983a,b; Hirel and Gadal, 1980).

Physiological studies employing ^{15}N labelling experiments with either intact cells (Betsche, 1983) or a reconstituted system (Ito et al., 1978; Keys et al., 1978) have been carried out to investigate the possible physiological roles of both forms of GS. Keys et al. (1978) proposed that cytosolic GS was exclusively involved in the reassimilation of the ammonia released in photorespiration while the chloroplast form functioned mainly in primary ammonia assimilation. However, a recent study has shown (McNally et al., 1983a) that the green leaf tissue of many higher plants contains only the GS_2 isoform and therefore refutes the unique involvement of GS_1 in the photorespiratory pathway and demonstrates unequivocally that it must be the chloroplastic enzyme which is responsible for the reassimilation of released ammonia during the decarboxylation of glycine to serine in photorespiration as has been shown to occur for spinach (Spinacia oleracea L.) (Woo and Osmund, 1982) and oats (Avena sativa L.)(Betsche, 1983). It has therefore been suggested (Hirel and Gadal, 1980) that cytosolic GS plays a role in the assimilation of ammonia in the dark.

Recently, it has been shown (McNally et al., 1983a) that higher plant species can differ in their GS isoform complement, and the physiological significance of this is still unclear. A group of plants which possess a modified nitrogen nutrition are stem and root obligate parasitic species of the Convolvulaceae, Viscaceae, Scrophulariaceae and Orobanchaceae. Members of the Scrophulariaceae and Orobanchaceae are important parasitic weeds on economically important crop plant species such as millet (Pennisetum americanum (L.) K. Schum) and sorghum and a variety of legumes. In the present paper we have investigated the occurrence and characteristics of glutamine synthetase isoenzymes in some angiosperm parasites, known to be dependent upon their hosts for their nitrogen nutrition (Mussleman, 1980).

MATERIALS AND METHODS

Plant material

Striga hermonthica (Del.) Benth. (host-millet) and S. gesnerioides (Willd.) Vatke. (host-Indigofera-hirsuta L.) were grown under greenhouse conditions; the temperature was maintained between 30-35°C. Pots of 15 cm diameter were half filled with John Innes Compost number 2, the top 6.0 cm layer of soil was first mixed uniformly with the appropriate parasitic seed species and then added to the pots. One crop seed per pot was pushed to 0.5 cm depth of compost. Orobanche minor Smith (host-Trifolium repens L.), O. hederae Duby (host-Hedera helix L.), O. cernua Loefl. (host-Senecio sp.) were grown in the same way as detailed for Striga spp. except that the temperature was maintained between 18-25°C. Lathraea spp. were obtained from the Botanical Supply Unit, Royal Holloway College, Egham, Surrey. Arceuthobium oxycedri (D.C.) M. Bieb. was kindly provided by Peter Wyse-Jackson (Botanic Gardens, Trinity College, Dublin). Melampyrum arvense L. was collected from Lathkill Dale in Derbyshire.

Enzyme extraction and ion-exchange chromatography

All operations were carried out at 4°C. Plant tissue was ground in a pestle and mortar with 2% Polyclar AT and extraction buffer consisting of 50 mM Tris-HCl, except A. oxycedri (12.5 mM Tris-HCl buffer), pH 8.0 (pH 7.6 for Striga spp.) containing 1.5% (w/v) soluble PVP; 1.0 mM EDTA; 1.0 mM DTT, 1.0 mM reduced glutathione; 10 mM magnesium sulphate and 5.0 mM glutamate. B-mercaptoethanol was included at 1.0 mM for all species except Striga, Orobanche and Arceuthobium (20 mM). The homogenate was filtered through muslin and centrifuged for 15 min. at 4200 rev. min.$^{-1}$ in a MSE Chillspin equipped with a swing-out rotor. The supernatant (crude extract) was first desalted on a G25 sephadex column, pre-equilibrated with extraction buffer minus Polyclar AT and soluble PVP, and then applied to a DEAE sephacel (Pharmacia Ltd.) column (10 cm x 1.0 cm) equilibrated with extraction buffer. Elution was carried out as described by McNally et al. (1983a).

Enzyme assays

Determination of transferase and synthetase activities of glutamine synthetase were as described by Rhodes et al. (1975).

Immunoprecipitation

Antibodies against the cytoplasmic glutamine synthetase of barley (Hordeum vulgare L.) and the chloroplastic glutamine synthetase of tobacco (Nicotiana tabacum L.) were obtained as described by Hirel et al. (1982). Immunoprecipitation was carried out as described by McNally et al. (1983a).

Amino acid analyses

Amino acids in the xylem sap of Striga (host-millet) were determined by gas chromatography of their N-heptafluorobutyryl isobutyl esters using a Varian 3700 gas chromatograph fitted with a fused silica capillary column of OV-101. The procedure employed for derivatization and chromatography were as described by Rhodes et al. (1981).

RESULTS

Chromatography on DEAE-sephacel of an extract of green leaf tissue of the stem parasite A. oxycedri shows the presence of two peaks of glutamine synthetase activity (Fig. 1). The first peak eluted at 100 mM KCl while the second eluted at 240 mM KCl. These peaks are designated GS_1 and GS_2 respectively. Table 1 shows the total GS activity and also the relative proportions of GS_1 and GS_2 which are expressed as a percentage of the total activity of glutamine synthetase after ion exchange chromatography of either the leaf or shoot tissue of the parasitic species studied.

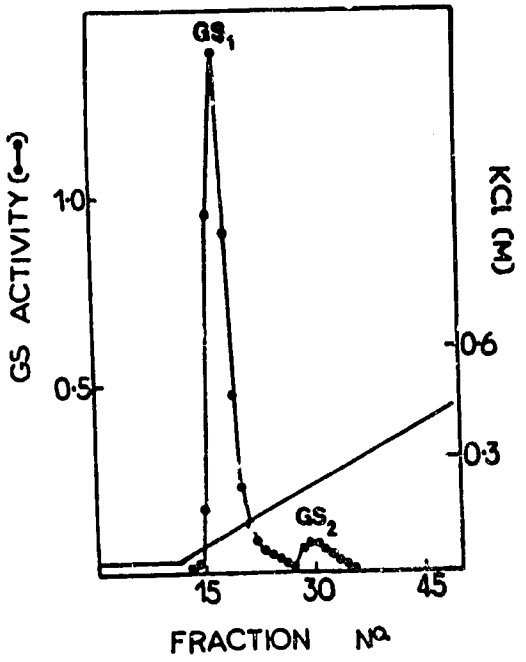

Fig. 1. Elution profile of GS_1 and GS_2 of the chlorophyllous stem parasite, A. oxycedri, after chromatography on DEAE-sephacel ion-exchange resin. Recovery of GS activity after chromatography was greater than 90% of the total activity initially loaded onto the column. Maximum synthetase activity was 0.52 µmol min^{-1} gfw^{-1}

Table 1

Glutamine Synthetase Activity of Higher Plant Parasites

Plant Species		Total GS activity[a]	% Activity GS_1	GS_2
Cuscuta australis	(NPSP)[b]	12.5	100	0
Arceuthobium oxycedri	(PSP)	13.1	>90	<10
Orobanche minor	(NPRP)	15.6	100	0
Orobanche hederae	(NPRP)	17.1	100	0
Orobanche cernua	(NPRP)	17.8	100	0
Orobanche ramosa	(NPRP)	16.3	100	0
Lathraea squamaria	(NPRP)	13.6	100	0
Lathraea clandestina	(NPRP)	14.7	100	0
Striga hermonthica	(PRP)	18.9	>90	<10
Striga gesnerioides	(PRP)	16.0	>90	<10
Melampyrum arvense	(PRHP)	-	<30	>70
Etiolated Striga hermonthica		5.0	100	0

[a] Transferase Activity (µmol min^{-1} gfw^{-1})

[b] NPSP, non-photosynthetic stem parasite
PSP, photosynthetic stem parasite
NPRP, non-photosynthetic root parasite
PRP, photosynthetic root parasite
PRHP, photosynthetic root hemi-parasite

Immunoprecipitation experiments were employed to characterize unambiguouly the GS_1 and the GS_2 isoforms from some of the parasitic plant species using specific antibodies raised against the cytosolic GS from barley and chloroplastic GS from tobacco. GS_1 and GS_2 were separated by ion exchange chromatography on DEAE-sephacel and immunoprecipitated. Constant amounts of enzyme were incubated with increasing concentrations of antibodies and the enzyme activity was measured in the supernatant following the removal of the immune complexes. Figure 2 shows the results obtained with the chlorophyllous stem parasite, A. oxycedri; similar results were observed with GS_1 and GS_2 isolated from the leaf or shoot tissue of the other parasitic plant species. GS_1 is completely precipitated with 100 µl of GS_1 antibodies and likewise GS_2 with 100 µl of GS_2 antibodies. However, only 40% of the total enzyme activity of GS_2 was precipitated with 100 µl GS_1 antibodies and only 50% of the total enzyme activity of GS_1 was precipitated with 100 µl GS_2 antibodies.

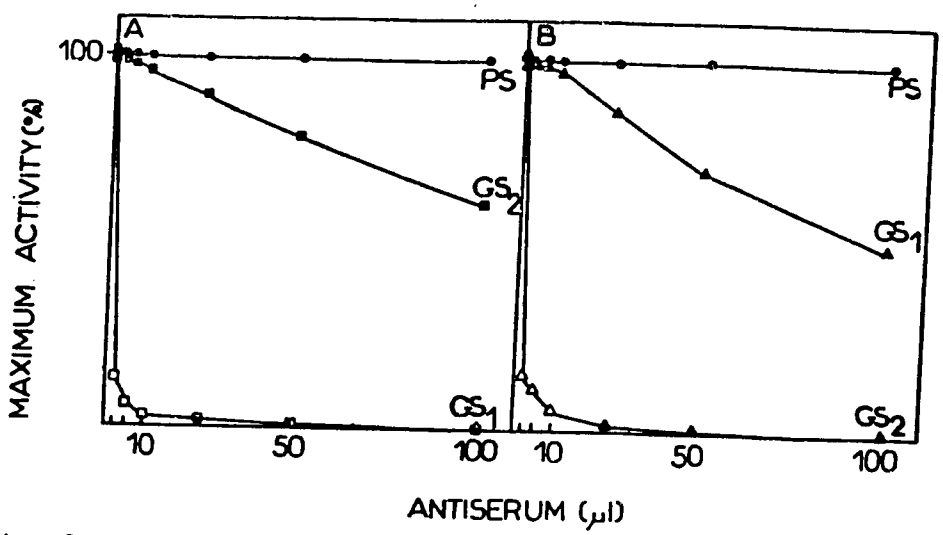

Fig. 2. Immunoprecipitation curves of GS_1 and GS_2 from the chlorophyllous stem parasite, A. oxycedri. In A and B GS_1 and GS_2 were separated by using DEAE-sephacel chromatography and submitted separately to immunoprecipitation with antibodies raised against either barley cytosolic GS (A) or tobacco chloroplastic GS (B). Constant amounts of GS_1 and GS_2 activity (0.85 nkatal) were incubated with increasing volumes of either antiserum or non-immune serum. Samples were incubated for 3 h at 20°C and then centrifuged at 10,000 g for 15 min. GS activity was assayed in the supernatant. A: GS_1 (□) and GS_2 (■) from A. oxycedri incubated with barley GS_1 antiserum. B: GS_1 (▲) and GS_2 (△) from A. oxycedri incubated with tobacco GS_2 antiserum. A & B: control with non-immune serum (●).

DISCUSSION

Chromatography of the green leaf tissue of the hemi-parasite, M. arvense, and the obligate parasites, A. oxycedri, S. hermonthica and S. osnerioides revealed two distinct peaks of GS activity which correspond to the cytoplasmic (GS_1) and chloroplastic (GS_2) isoenzymes of glutamine synthetase found in other higher plants (Mann et al., 1979; Hirel and Gadal,

1980; Hirel and Gadal, 1982; Ahmad et al., 1983). Moreover, the isoforms detected after chromatography of either green leaf or shoot tissue were unambiguously identified using specific antibodies raised against purified GS_1 and GS_2. The results obtained here show that the photosynthetic hemi- and obligate parasites possess both cytosolic and chloroplastic isoforms. However, in both species of Striga and A. oxycedri the activity of the chloroplastic GS is much lower (<10% of total GS activity) than in the hemi-parasite, M. arvense (>70% of total GS activity). In all the achlorophyl-lous parasites studied only cytosolic GS was present.

The presence of the GS_2 isoform and its relative proportion of total GS activity within green leaf tissue seems to be strongly correlated with photosynthetic activity. For example, in S. hermonthica, the rate of photo-synthesis and the amount of GS activity present in the chloroplast is much lower than in a typical C_3 plant. Moreover, in achlorophyllous stem and root parasites which do not photosynthesize, GS_2 is absent as found in etio-lated tissue of several other higher plants (Mann et al., 1980 and Hirel and Gadal, 1982) including, in the present study, etiolated Striga.

The proposal of the unique functioning of GS_1 in the reassimilation of ammonia released during photorespiration is not substantiated by the present findings. Achlorophyllous parasites which do not photosynthesize and which are unlikely to photorespire paradoxically possess only the GS_1 isoform. Moreover, in S. hermonthica it is likely that chloroplastic GS is the iso-form responsible for the reassimilation of ammonia released during photo-respiration which has been already found to occur in other C_3 plants such as spinach (Woo and Osmund, 1982) and oats (Betsche, 1983). Interpretat-ions concerning the role of cytosolic GS are still speculative. However, there is increasing evidence to support its functioning in the assimilation of ammonia in the dark, a situation comparable to that already found in non-photosynthetic organs such as roots (Mann et al., 1980) and seeds (Hirel et al., 1984) and also in the shoot tissue of achlorophyllous parasites.

There is evidence to support the use of fertilizer nitrogen for Striga control in cereals (Gambia, 1981). The application of organic manure to infested stands reduced the emergence of Striga and increased crop yield. The nitrogen acts in at least two ways: indirectly by reducing the light intensity at the soil surface as a result of denser crop canopy (Ogborn, 1983) and systematically by increasing tissue nitrogen of the host root to a level at which Striga parasitism is directly inhibited (McNally and Stewart, 1982). In this context it is of interest to find a very low nitrate concen-tration (0.059 mM; at least ten times lower than that of its host, millet) in the xylem sap of Striga, this together with a very low chloroplastic GS activity are in favour of a very low primary assimilation of ammonia released from nitrate reduction. This could also explain the presence of large amounts of organic nitrogen in the form of glutamic acid (1430 nmol cm^3) in the xylem sap exudate of Striga. Glutamic acid is likely, therefore, to be the major source of nitrogen transported from the millet host to the Striga parasite, a situation previously reported for the Alnus-Lathraea parasitic association (Renaudin and Lahrer, 1982) where glutamic acid and citrulline are the major transport amino acids. Therefore it appears that

organic nitrogen compounds are preferentially transported from host to parasite as opposed to inorganic forms of nitrogen.

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A BIOCHEMICAL CHARACTERIZATION OF STRIGA HERMONTHICA

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Abstract. The parasitic plant Striga hermonthica (Del.) Benth is shown to have a low capacity for CO_2 fixation and nitrate reduction. The activities of Ribulose bis-phosphate carboxylase, nitrate reductase and chloroplastic glutamine synthetase are low. The low levels of RUBP carboxylase activity are consistent with the low rates of photosynthesis, and the low levels of chloroplastic glutamine synthetase with low photorespiratory activity. Analyses of the xylem sap of S. hermonthica reveal the presence of a range of amino acids and amides, suggesting that some amino acids may be derived directly from the host rather than by synthesis in the cells of Striga. The dependance of Striga on its host for organic carbon and nitrogen-containing compounds may explain in part its parasitic mode of nutrition.

INTRODUCTION

Under field conditions Striga hermonthica (Del.) Benth is always found parasitizing the roots of other plants. In culture, however, it can be grown on a relatively simple defined growth medium (Okonkwo, 1966). Such studies have led to the assumption that it depends on its host for minerals, water and possibly also hormonal compounds (see Musselman, 1980). One implication of these conclusions is that its biosynthetic potential is similar to other free living angiosperms. Our contention is that axenic culture studies do not provide us with any information on the host dependence of the parasite or on its "normal" biochemical capacity. In this paper we present results on the biochemical status of plants of Striga parasitizing sorghum which suggest a high degree of dependence on the host for organic carbon and nitrogen containing compounds.

MATERIALS AND METHODS

Materials

S. hermonthica and S. asiatica were grown as described previously (McNally et al, 1983a), on Sorghum bicolor (L.). Moench var Ras Girid as the host. S. gesnerioides was grown on Indgofera hirsuta as host.

Enzyme Extraction and Assay

Shoot tissue of Striga species was extracted in 0.1M Tris-HCl containing 1mM EDTA and 1mM dithiothreitol using a polytron homogenizer. Homogenates were centrifuged at 5,000 g for ten minutes and the supernatant used as a source of enzyme. Nitrate reductase was assayed as described by Stewart and Orebamjo (1980). RUBP carboxylase was assayed as described by Lilly and Walker (1975). Glutamine synthetase, glutamate synthase and glutamate dehydrogenase assays were according to Rhodes et al, (1975), ion-exchange chromatography of glutamine synthetase was carried out as before (McNally et al, 1983a). PEP-carboxylase assay was according to Smith and ap Rees (1979).

Amino Acid Analyses

Amino acids were extracted, fractionated, derivitized and determined as described by Rhodes et al (1981).

Gel Electrophoresis

12% SDS-page (Laemelli system) gels were used. Samples were prepared by grinding shoot tissue in a 1.25% mercaptoethanol, 0.125M Tris-Cl extraction buffer, acid precipitating the supernatant and resuspending the precipitate in a glycerol based sample buffer.

Measurements of photosynthesis and photorespiration were carried out by Dr. P.J. Lea and his colleagues, Biochemistry Department, Rothamsted.

RESULTS

Enzymes of Inorganic Nitrogen Assimilation

The results in Table 1 show the activity of enzymes of inorganic nitrogen metabolism in leaf or shoot tissue of Striga species. Very low levels of nitrate reductase were present; the activity measured is comparable with that found in plants of Lemna minor L. assimilating nitrogen sources other than nitrate (see Orebamjo & Stewart, 1974). The activity of cytoplasmic glutamine synthetase is similar to that in many non parasitic angiosperms (see McNally et al, 1983b) but the activity of chloroplastic glutamine synthetase is very low and is comparable with that in etiolated tissue of rice (Guiz et al, 1979).

Table 1. Enzymes of nitrogen assimilation in Striga Species

Enzyme	Enzyme Activity $\mu mol\ h^{-1} gfw^{-1}$		
	S. asiatica	S. hermonthica	S. gesnerioides
Nitrate reductase	0.2	0.1	0.1
Glutamine synthetase (cytosolic)	29.5	33.0	26.0
Glutamine synthetase (Chloroplastic)	1.5	9.0	5.0
Glutamate synthase	7.3	20.0	12.3
Glutamate dehydrogenase	17.0	26.0	21.0

Nitrate reductase is a substrate-inducible enzyme (see Hewitt, 1975) so that the low activities in Striga spp. could reflect a limited transport of nitrate in the xylem sap. However, feeding nitrate to cut shoots of S. hermonthica did not bring about an induction of nitrate reductase. Ferredoxin dependent glutamate synthase was detectable but again its activity is relatively low (cf Lee & Stewart, 1978). The activity of glutamate dehydrogenase is within the range found for many other non-parasitic angiosperms (cf Lee & Stewart, 1978).

The low capacity of inorganic nitrogen assimilation and amino acid biosynthesis suggests parasitic plants of Striga may utilize a source of nitrogen other than nitrate. Analyses of the xylem sap of S. hermonthica (Table 2) show that very low amounts of nitrate are present and that a wide variety of amino acids are present. Although the main components are aspartic acid, asparagine, glutamic acid and glutamine it is evident that other amino acids are being supplied to the shoot. These analyses suggest that parasitic plants of Striga receive a number of protein amino acids directly from the host. It is possible, however, that interconversion of nitrogenous compounds may occur in the haustoria and that some of the xylem nitrogenous compounds derive from the biosynthetic activity of the parasite rather than its host.

Table 2. Nitrogenous compounds in the xylem sap of *Striga hermonthica* parasitizing Sorghum

Compound	nmol cm^3
Nitrate	15
Asparagine	440
Aspartic acid	470
Glutamic acid	410
Glutamine	290
Isoleucine	28
Leucine	21
Lysine	76
Phenylalanine	120
Proline	55
Serine	14
Threonine	40
Valine	40

CO_2 fixation

Compared with other non-parasitic species in the Scrophulariaceae the extractable activity from *S. hermonthica* of RUBP carboxylase is low (Table 3). The levels of the alternative carboxylating system PEP-carboxylase was 0.1 mol m^{-1} gfw^{-1}, a value comparable with other species exhibiting C_3 photosynthesis.

Table 3. Ribulose bis-phosphate carboxylase activity of *Striga* and other non parasitic members of the Scrophulariaceae.

Species	µmol h^{-1} mg^{-1} chlorophyll	µ mol h^{-1} mg^{-1} protein
Striga hermonthica	0.2	1.5
Digitalis purpurea L.	4.43	4.0
Linaria vulgaris Mill.	3.5	5.4
Antirrhinum majus L.	11.4	11.5

The low levels of extractable RUBP carboxylase appear to reflect the presence of small amounts of this protein in leaf tissue of *Striga*. Thus on SDS-polyacrylamide gel electrophoresis of *Striga* leaf extracts the protein which corresponds to the large sub-unit of RUBP-carboxylase gives a faintly staining band in comparison with that in extracts from non-parasitic species.

Measurements of photosynthesis and photorespiration gave rates of 5.5mg CO_2 $dm^{-2}h^{-1}$ and 1.8mg $CO_2dm^{-2}h^{-1}$ respectively which are low in comparison with those reported for other species (e.g. barley which under similar conditions gave rates of 26.5 and 5.3 mg $CO_2dm^{-2}h^{-1}$) and are consistent with the low levels of RUBP carboxylase.

DISCUSSION

It is often assumed that since hemi-parasites are chlorophyll-containing and have apparently normal chloroplast lamella structure, they are not dependent on their host for carbon compounds. The present results suggest that parasitic plants of Striga have a low capacity for photosynthesis, arising partly at least from the low activity of RUBP carboxylase. The low rates of photosynthesis are matched by low rates of photorespiration. This may in part explain the low levels of chloroplastic glutamine synthetase reported previously (McNally et al, 1983a). Although Keys et al (1978) suggested in their model of the photorespiratory nitrogen cycle (PRNC) that the ammonia released in photorespiration is reassimilated by cytoplasmic glutamine synthetase, results obtained more recently (see McNally et al, 1983b) suggest it is the chloroplastic glutamine synthetase which is active in the PRNC. The low levels of chloroplastic glutamine synthetase present in Striga plants exhibiting low rates of photorespiration are consistent with this interpretation.

The absence of any marked capacity to reduce nitrate indicates alternative nitrogen sources are utilized and analyses of the xylem sap suggest amino acids and amides are these nitrogen sources. The transport of organic nitrogen containing compounds has important implications as regards the carbon relations of the parasitic association. Raven (1983) has drawn attention to the considerable "obligate" transfer of carbon from host to parasite when nitrogenous compounds are translocated from host to parasite. The carbon:nitrogen ratio in Striga shoots is approximately 20:1 while that of the nitrogenous compounds in the xylem sap is approximately 3:1. These values imply that up to 17% of the carbon in Striga shoots could be derived through the obligate transfer of carbon during the transport of nitrogenous solutes from host to parasite. If, however, the haustorial cells are active in nitrogen assimilation the input of host carbon may in fact be less than calculated. The low rates of photosynthesis observed here suggest more detailed studies of carbon transfer between host and parasite would be worthwhile. Raven (1983) has suggested that the extent of carbon transfer could be determined by measuring the carbon isotope ratio. Plants possessing the C_4 photosynthetic pathway have a lower $\delta^{13}C$ value than plants with only the C_3 pathway (O'Leary, 1981). Consequently any

substantial input of carbon derived from sorghum photosynthate will result in a less negative $\delta^{13}C$ value for Striga compared with other C_3 species. The $\delta^{13}C$ values obtained for Striga bilabiata (Thunb.) Kuntz. parasitizing Eragrostis racemosa (Thunb.) Steud.) was -25.4 compared with -11.6 for the C_4 host (de La Harpe et al, 1981) suggesting little carbon transfer occurs between host and parasite in this particular association.

Although the capacity of Striga to grow in axenic culture on a relatively simple growth medium implies it can assimilate C and N, the present results suggest parasitic plants have a high dependence on the host for assimilated carbon and nitrogen compounds. It may be that there are qualitative differences between parasitic and autotrophic plants of Striga.

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THE ROLE OF MANNITOL IN STRIGA HERMONTHICA AND OTHER PARASITIC ANGIOSPERMS.

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Abstract. The polyol, mannitol is present in large amounts in *Striga hermonthica* (Del.) Benth. and several other parasitic angiosperms. The occurrence of large quantities of potassium ions in leaves of *Striga* suggests that mannitol could have an osmoregulatory function. In addition, mannitol is shown to increase the heat resistance of enzymes and membranes indicating it could also have a protective role.

INTRODUCTION

Several parasitic angiosperms, members of the Orobanchaceae and Scrophulariaceae are reported to contain the sugar alcohol, mannitol (Smith *et al.*, 1969). Sugar alcohols (polyols) such as sorbitol (Ahmad *et al.*, 1979), mannitol and pinitol (Popp, 1984) are present in large amounts in several species of angiosperm halophytes, particularly species of mangrove. In the latter group of plants sugar alcohols (or polyols) are suggested to play a role in osmotic adaptation (Ahmad *et al.*, 1979) and in non-halophytes they are suggested to act as macromolecule protectants (Smirnoff and Stewart, 1984). The role of mannitol in angiosperm parasites is uncertain. Smith *et al.*, (1969) propose that angiosperm parasites which derive their carbon predominantly from their host conform to the generalization, applicable to many symbiotic associations, that the parasite synthesizes soluble carbohydrates which differ from those of the host. Mannitol would then represent a carbohydrate source not readily re-utilised by the host. Smith *et al.*, (1969) also suggest polyols might play a role in the water relations of parasitic angiosperms, providing a source of osmotically active solute in order to reduce the water potential of the parasite below that of its host.

In this paper we consider the possible role of mannitol as an osmotic solute and macromolecule protectant.

MATERIALS AND METHODS

Plant samples: Striga was grown as described previously (McNally et al., 1983). Striga hermonthica (Del.) Benth. from sorghum host in Abu Naama, Sudan was grown on sorghum (Sorghum bicolor) (L.) Moench. Plants of Lathraea squamaria L. were collected from CressBrook Dale, Derbyshire. Orobanche elatior Sutton and O. hederae Duby were grown on plants of Centaurea scabiosa L. and Hedera helix L. respectively. Cuscuta australis R. Brown was grown on a range of host species (see results).

Mannitol Extraction and Estimation

Plant samples were ground to a fine powder and extracted in a methanol (12):chloroform (5):water (3) mixture. After phase separation and evaporation to dryness the sample was taken up in water and applied to a column of Dowex-50 H^+. Sugars and organic acids were recovered in the water wash and applied to a column of Dowex-1 formate. The sugars were recovered in the water wash, which was taken to dryness and redissolved in a small volume of water. An aliquot of this fraction was taken to dryness in a reactivial, 100 µl of STOX reagent (Pierce-Warriner) was added and the mixture incubated at 70° for 30 minutes. 100 µl of Bis-trimethylsilylacetimide was added. After at least 60 minutes samples were taken for analysis by capillary gas chromatography on a Varian 3700 gas chromatograph fitted with a fused silica capillary column of OV-101. GC conditions were; isothermal operation 150°; injector and detector temperatures 280°C; with helium as the carrier gas.

Enzyme Extraction and Assay

The extraction of enzymes from shoot tissue of S. hermonthica was as described by McNally et al. (1983). Details of the enzyme assays employed are given in Stewart and Lee (1972) and Rhodes and Stewart (1974).

Mineral Analysis: Dried samples were used for cation determinations (Ahmad et al., 1979).

RESULTS

Mannitol Content and Distribution

The mannitol content of Striga and some other angiosperm parasites is shown in Table 1. The presence of mannitol in Orobanche and Lathraea confirm the results of earlier studies (see Smith et al., 1969), and the occurrence of mannitol in Striga is consistent with reports of its presence in other parasitic species of the Scrophulariaceae (see Smith et al.,

1969). Mannitol was present in substantial amounts in all the tissues of Lathraea examined. It is evident from the results that the presence of large amounts of mannitol in Cuscuta is host-dependent. Only in C. australis parasitising Terminalia argentea and S. hermonthica was mannitol present in large amounts. Both these hosts accumulate mannitol. More detailed studies of Cuscuta have shown its soluble carbohydrate composition to be closely related to that of its host.

Table 1

Mannitol Concentrations in some Parasitic Angiosperms

SPECIES		Mannitol $\mu mol\ gfw^{-1}$
Lathraea squamaria		20-30
Orobanche elatior		17-26
Orobanche hederae		25-35
Striga asiatica		10-15
Striga hermonthica		10-20
Cuscuta australis	(on Avicennia nitida Jacq.)	N.D.
	(on Cicer arietinum L.)	N.D.
	(on Ricinus communis L.)	N.D.
	(on Striga hermonthica)	20
	(on Terminalia argentea)	12

Osmotic Adjustment and Mannitol

Analyses of S. hermonthica indicate the presence of large amounts of potassium and magnesium (Table 2). The occurrence of large quantities of potassium (up to five times those regarded as being "adequate" for plant growth - Epstein, 1972) is of interest in relation to its suggested role in osmoregulation (Cram, 1976; Wyn Jones et al., 1979). The concentrations of potassium in S. hermonthica are in excess of 300mM and as such must make a major contribution to leaf osmotic potential (>1.20 MPa). Wyn Jones et al., (1979) have proposed that the potassium concentrations in the cytoplasm of eukaryotic cells are maintained in the range 100-120mM. If it is assumed that the cytoplasm of a Striga cell

comprises approximately 10% of the total cell water and that cytoplasmic potassium is 100M then there will be a large difference in osmotic potential between the cytoplasm and vacuole. This possible difference in ion concentration between cytoplasm and vacuole suggested for Striga is reminiscent of a similar phenomenon in many angiosperm halophytes (see Flowers et al., 1977). Osmotic adjustment in angiosperm halophytes is achieved by the synthesis of cytoplasmic osmotic solutes, including polyol (see Wyn Jones et al., 1977; Stewart and Ahmad, 1983). It is possible that a similar role in osmoregulation can be ascribed to mannitol accumulation in Striga and other angiosperm parasites.

Table 2

Mineral Content of Striga hermonthica

ELEMENT	mg gdw^{-1}
Calcium	1.8
Iron	0.3
Magnesium	15.0
Manganese	0.1
Potassium	54.0
Zinc	0.1

Plants of Striga used in these analyses were parasitic on Sorghum bicolor and had just begun to flower.

The effect of mannitol on the stability of enzymes

Fig 1 shows the effect of mannitol on the heat stability of four enzymes from shoot tissue of S. hermonthica. It is evident from the results that mannitol increases the heat stability of all four enzymes. The temperature giving a 50% loss of activity is raised some 5-10° in the presence of mannitol.

This protective effect is dependent on solute concentration (Table 3). Over the range 0.1 to 1.0M, mannitol progressively increased the heat stability of glutamine synthetase from Striga. This stabilisation is not however species or solute specific. Mannitol is as effective in stabilising glutamine synthetase from a mannitol non-accumulator (Tortula ruraliformis (Besch.) Rich &

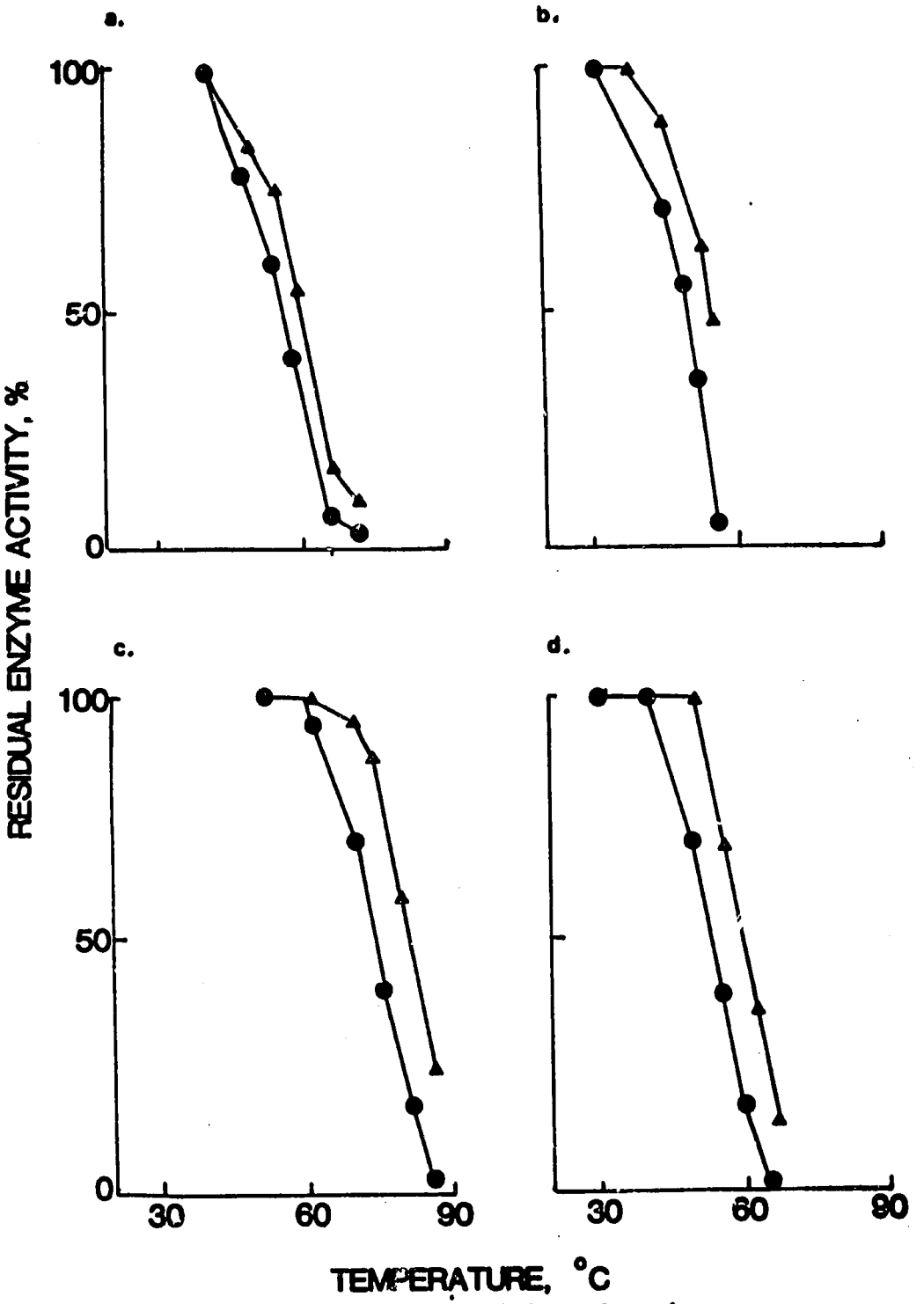

Fig. 1. Influence of mannitol on heat stability of Striga enzymes.
(a) Acid phosphatase, (b) Aspartate aminotransferase, (c) Glutamate dehydrogenase, (d) Malate dehydrogenase.
Partially purified enzymes were incubated at different temperatures for 10 minutes in presence or absence of 1.0M mannitol.
(● control, ▲ 1.0M mannitol)

Wall) as the Striga enzyme (see Smirnoff and Stewart, 1984).

Table 3

Effect of mannitol on heat stability of glutamine synthetase

Mannitol (M)	%Residual glutamine synthetase activity
0	30
0.1	40
0.25	47
0.5	56
1.0	65

Partially purified glutamine synthetase was incubated at 50° for 10 minutes in the presence of different concentrations of mannitol, aliquots were removed and residual activity determined.

Other solutes which accumulate in plants subject to various stresses, including proline and glycine betaine give a similar if somewhat lesser protection (Paleg et al., 1981; Smirnoff and Stewart, 1984).

The results in Fig. 2 show that increasing mannitol concentrations protect Striga glutamine synthetase against denaturation by high concentrations of urea. A related polyol, sorbitol was found to protect the glutamine synthetase of Triglochin maritima L. against salt, temperature and pH denaturation (Ahmad et al., 1979). These, and the results presented here suggest polyols and the compatible solutes proline and glycine betaine (see Paleg et al., 1981) have a generalised protective effect on enzymes.

Mannitol may protect cell membranes against heat denaturation. Incubating fragments of Striga leaves at increasing temperatures leads to a leakage of amino acids which is considerably reduced in the presence of 0.5M mannitol (Fig 3). This suggests mannitol may protect cell membranes against heat denaturation.

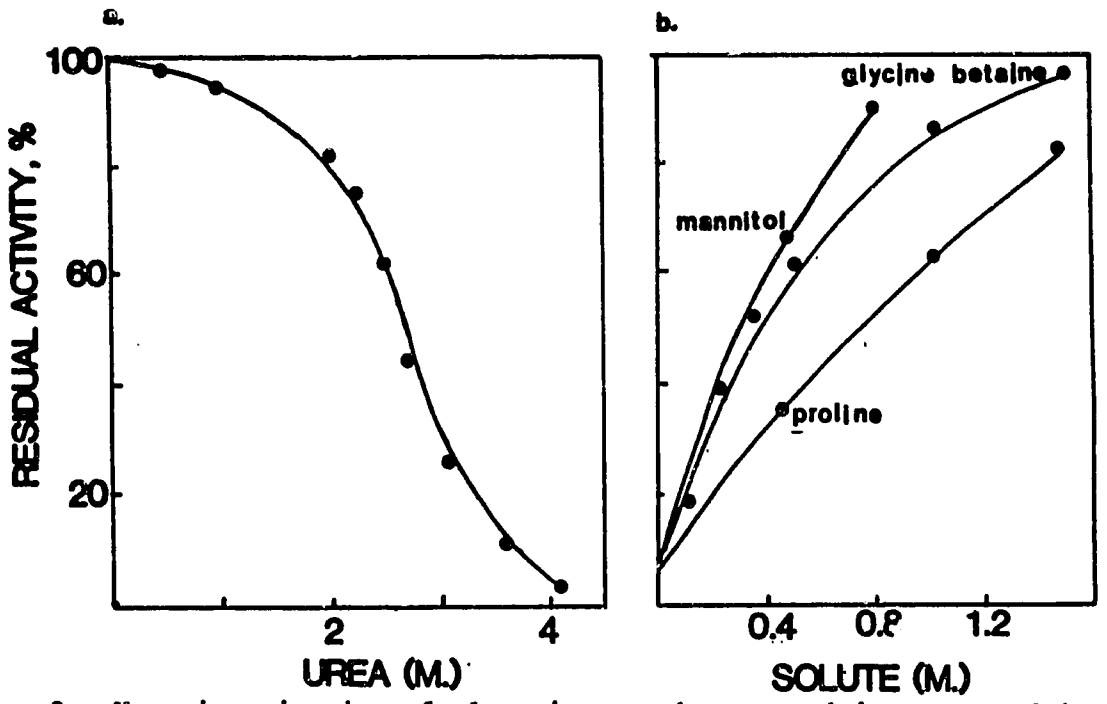

Fig. 2. Urea inactivation of glutamine synthetase and its reversal by mannitol. (a) Partially purified glutamine synthetase was incubated in the presence of increasing urea concentrations and samples removed to determine residual activity after 10 minutes. (b) Glutamine synthetase was incubated in the presence of 4M urea and different concentrations of proline, glycine betaine or mannitol.

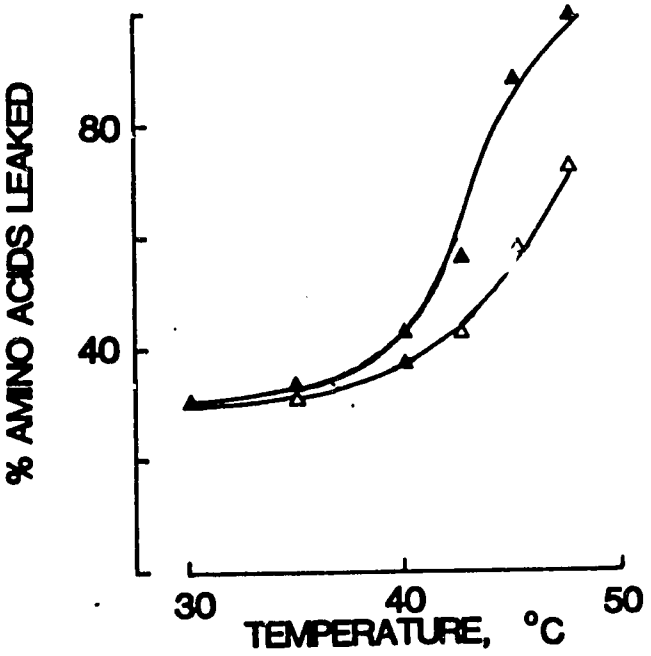

Fig. 3. Influence of mannitol on amino acid leakage from Striga leaves. Leaf fragments were incubated in the presence or absence of mannitol (0.5M) and aliquots removed after 30 minutes for the determination of amino acids (△ control, ▲ 0.5M mannitol).

DISCUSSION

Mannitol accumulation if it is predominantly located in the cytosol of Striga would seem to provide a mechanism for intracellular osmotic adjustment in cells accumulating large amounts of potassium. In addition mannitol is able to increase the in vitro heat stability of enzymes and to increase the heat stability of cell membranes. Studies with other compatible solutes, proline and glycine betaine have shown they can increase the heat stability of enzymes in chloroplasts and mitochondria isolated from leaves (Nash et al., 1982). Increased heat resistance in Striga could be of particular significance during the period before emergence when high soil temperatures might be encountered. Further experiments are necessary to investigate the influence of water stress, temperature and other parameters on the accumulation of mannitol in Striga.

Mannitol has not been detected in the hosts of S. hermonthica, sorghum or millet (Pennisetum americanum (L.) K. Schum.). The role of mannitol in osmoregulation and possibly also heat resistance suggests interference with its metabolism would have marked effects on Striga growth and reproduction. Mannitol metabolism would therefore seem to be a possible target for enzyme-directed herbicides. The occurrence of mannitol in other parasitic weeds such as Orobanche indicate the possibility for the development of a selective herbicide for these noxious weeds.

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THE INFLUENCE OF STRIGA SPECIES ON SORGHUM UNDER VARYING NITROGEN FERTILIZATION

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Abstract Sequential harvests of sorghum (*Sorghum bicolor* (L.) Moench) and associated parasite *Striga hermonthica* (Del.) Benth. at two levels of nitrogen fertilization, confirmed the potential for *S. hermonthica* to reduce shoot weight of its host even before its own emergence from the soil. The root:shoot ratio, and actual root dry weight of the host were increased as a result of *Striga* attack. At low N this effect followed the inhibition of shoots and could be an indirect effect resulting from passive diversion of resources to the host root system. At high N the development of *Striga* was delayed and the damage to the host considerably reduced. It is proposed that the effect of N can be at least partly explained by its tendency to influence the host's partitioning of resources between shoot and root. The damaging effects of *S. hermonthica*, *S. asiatica* (L.) Kuntze and *S. densiflora* Benth. appear to be directly related to the weight of emerged *Striga*, over a wide range of intensities of infection.

INTRODUCTION

Most authors including Saunders (1933), Uttaman (1950) etc. have concluded that the parasitic *Striga* species induce some toxic effect in their hosts, rather than merely depriving the host of nutrients and water. In work with *Striga* species spanning many years at the Weed Research Organization, and earlier at Oxford University (e.g. Parker, 1974) it had been quite apparent that there was indeed a toxic effect of *Striga* but it had also been observed with both *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze that while shoots of sorghum (*Sorghum bicolor* (L.) Moench) or other cereal hosts were conspicuously reduced, sometimes even before the emergence of *Striga* from the soil, the root systems were frequently larger.

Many authors have observed a beneficial effect of nitrogen in reducing *Striga* emergence and/or reducing the damage caused (Pearson, 1913; Last, 1960 etc.).

An experiment was therefore designed to observe the performance of both sorghum host and *S. hermonthica* over the early stages of *Striga* attack, and to see how the performance of both host and parasite were influenced by contrasting levels of nitrogen. The experiment was conducted in 1973 and results were presented orally at a meeting of the Association of Applied Biologists in 1975. Only a short abstract was published at that time (Parker, 1976) and the opportunity is taken here to record the full data, albeit belatedly, and to discuss the results in relation to some more recent work.

METHODS AND MATERIALS

These pot experiments were conducted in the glasshouses at WRO in the summer months without supplementary light but with heating to maintain a minimum 23°C. Ventilation was controlled and for the period from 2 to 4 weeks after sowing, temperatures were allowed to rise naturally under sunny conditions to 30-35°C in order to ensure good germination of *Striga*.

Sequential harvest experiment.

In this experiment, conducted in 1973, the soil used was a sandy loam from Begbroke Hill Farm. P and K fertilizers were added to all soil in the form of superphosphate at 1.2 g/kg soil and potassium sulphate at 0.6 g/kg. For the 'low' rate of N, ammonium sulphate was added at 0.1 g/kg and for the 'high' rate, at 1.6 g/kg. No further additions of fertilizer were made after this initial soil dressing.

Pots of 13 cm diameter were half filled with this soil. Seeds of *S. hermonthica* from a sorghum host at Samaru, N. Nigeria were then mixed into soil at approximately 1000 seeds per pot. This soil was used to fill the upper 6 cm of pots. Three seeds of hybrid sorghum var. MS 348 were sown 3 per pot 1-2 cm deep and thinned to one plant soon after emergence. Pots were watered individually as required.

At weekly intervals from 3 weeks to 8 weeks 5 replicate pots were taken from each of the four treatments and the following parameters measured: (a) maximum height from soil level to tip of longest leaf, (b) height of the stem apex from soil level (observed after cutting the shoot and splitting up from the base), (c) number of leaves visible, (d) degree of senescence of lower leaves, (e) stage of development of the inflorescence, (f) leaf area, measured by laying individual leaves on a light screen, photographing and measuring light transmission through the negatives in a suitably calibrated photometer, (g) dry weight of shoots, (h) dry weight of roots, (i) number of prop roots longer than 1 cm, (j) number of *Striga* emerged, (k) number of *Striga* attached to roots, (i) dry weight of *Striga*.

Additional treatments in this experiment included overall sprays of growth regulators, which were applied 3 weeks after sowing using the WRO pot sprayer delivering 400 l/ha, with additional wetting agent ('Agral 90') at 0.2% w/v. Chlormequat (CCC) was used as the 40% commercial formulation 'Cycocel' and gibberellic acid was in the form of 100 mg 'Berelex Minor' tablets (mainly GA_3). These treatments and a corresponding control were replicated 3 times.

Varietal experiment

In this more recent experiment (1983) a similar sandy loam (Yarnton) soil was used with additional superphosphate (2 g/kg) and potassium sulphate (0.6 g/kg) and 'low' nitrogen (0.1 g ammonium sulphate/kg). Larger pots (15 cm diameter) were used in which *Striga* seed was mixed with

the upper 6 cm of soil. The experiment involved 8 varieties of sorghum but the results for only three susceptible varieties are presented. Each sorghum variety was established (3 per pot initially, thinned to 1) in pots without *Striga* and with 4 replicates each of 11 different collections of *Striga*, 2 samples of *S. asiatica*, 1 of *S. densiflora* Benth. and 8 of *S. hermonthica*, from various origins as indicated in Table 3.

Assessments included *Striga* emergence at weekly intervals, and finally at 15 weeks, fresh weights of sorghum and *Striga* shoot growth.

RESULTS

Sequential harvest experiment

Results presented in Table 1. show that *Striga* was already developing on the roots of low N plants 4 weeks from sowing and beginning to emerge at 5 weeks. The number emerged represented only 9% of the total number attached at 6 weeks and still only 17% at 8 weeks. High N delayed *Striga* development by one week, but numbers attached and their total dry weight were eventually higher than at low N. Percentage emergence of those attached was only 8% at 8 weeks.

Dry weights of sorghum shoots were already signigicantly reduced by *Striga* attack at low N 5 weeks after sowing, before *Striga* had started emerging in 4 of the 5 replicates. Reduction of shoot growth became progressively more marked at each harvest to finish at only 49% of uninfested controls. At high N, reduction in sorghum shoot growth due to *Striga* was delayed and became significant only at 7 weeks.

Sorghum root weights were not significantly affected by *Striga* at 4 weeks. At low N there was an apparent reduction due to *Striga* at 5 weeks. The 6 week harvest unfortunately had to be discarded due to inadequate washing. By 7 weeks there was a highly significant increase in root weight due to *Striga*, but after this there was little further root growth due to the severe damage suffered by the shoots at this stage. At high N, there was already a significant increase in sorghum root weight due to *Striga* at 5 weeks, which remained apparent through to 8 weeks. Not only was total root weight increased but the numbers of prop roots were also increased by at least 10% by *Striga* at both levels of N, (data not presented).

As a consequence of reduced shoot growth and stimulated root growth there was an approximate doubling of the root:shoot ratio as a result of *Striga* infection at both low and high N. The ratio was significantly reduced by high N, in both the presence and absence of *Striga*.

The reduction in sorghum shoot weight by *Striga* was associated with a reduction in plant height which was almost completely accounted for by reduction in stem length, the reduction being significant by 5 weeks at both low and high N. Leaf number and length were relatively unaffected and leaf area (data not presented) were reduced non-significantly, by less than 10% in each case.

Table 1

Performance of sorghum and Striga hermonthica at two levels of nitrogen fertilization

	Age in weeks at harvest					
	3	4	5	6	7	8
Striga emerged (No/pot)						
Low N : + Striga	0	0	0.2	7.8	12.2	13.6
(log)			(0.06)	(0.92)	(1.12)	(1.13)
High N: + Striga	0	0	0	0.6	2.0	8.4
(log)				(0.12)	(0.34)	(0.86)
S.E.	-	-		(0.34)		
Striga total (No/pot)						
Low N : + Striga	0	10.0	28.8	88.8	72.0	80.6
(log)			(1.45)	(1.93)	(1.85)	(1.85)
High N: + Striga	0	0	7.2	34.6	75.8	108.6
(log)			(0.82)	(1.52)	(1.87)	(2.2)
S.E.	-	-		(0.08)		
Striga dry wt (mg/pot)						
Low N : + Striga	0	0.3	13.5	31.9	54.4	91.9
High N: + Striga	0	0	2.2	15.9	48.5	116.0
S.E.	-	-	-	23.4		
Sorghum shoot dry wt (g/pot)						
Low N : No Striga	0.65	1.57	2.64	3.26	4.08	5.58
Low N : + Striga	0.64	1.18	1.86	2.34	2.54	2.78
Low N : (+ Striga as % of None)	(97.6)	(75.5)	(70.5)	(71.6)	(62.2)	(48.9)
High N: No Striga	0.71	1.83	4.27	6.59	9.87	13.84
High N: + Striga	0.73	1.69	4.09	6.22	7.60	8.75
High N: (+ Striga as % of None)	(102.0)	(92.5)	(95.9)	(93.8)	(77.0)	(63.0)
S.E.				0.25		
Sorghum root dry wt (g/pot)						
Low N : No Striga	0.03	0.49	0.92	-	1.00	1.53
Low N : + Striga	0.03	0.47	0.74	-	1.46	1.48
High N: No Striga	0.03	0.43	0.92	-	2.00	2.89
High N: + Striga	0.03	0.39	1.25	-	3.00	3.58
S.E.				0.12		
Sorghum root:shoot ratio						
Low N : No Striga	0.05	0.35	0.37	-	0.25	0.28
Low N : + Striga	0.05	0.39	0.40	-	0.61	0.54
High N: No Striga	0.04	0.22	0.22	-	0.20	0.21
High N: + Striga	0.04	0.22	0.31		0.40	0.41
S.E.				0.10		
Sorghum Ht of stem apex (cm)						
Low N : No Striga	-	15.4	41.6	57.6	75.4	89.6
Low N : + Striga	-	12.6	30.2	46.0	43.0	41.0
High N: No Striga	-	18.6	51.0	85.4	109.4	147.0
High N: + Striga	-	18.2	43.8	72.0	90.8	122.4
S.E.	-			3.8		

Table 2.

Some effects of GA and chlormequat on the growth of sorghum with and without Striga hermonthica, all at low N.

	No spray	Chlormequat 2.5 kg/ha	GA 0.4 kg/ha	S.E.
Sorghum foliage dry wt.(g/pot)				
No Striga	5.49	4.79	6.60	
Plus Striga	2.55	2.04	3.25	1.0
Height of sorghum stem apex (cm)				
No Striga	119.0	102.3	139.7	
Plus Striga	54.3	35.0	103.3	9.3
Sorghum root:shoot ratio				
No Striga	0.247	0.280	0.169	0.014
Plus Striga		not recorded		

Table 2 shows that GA treatment had opposite effects to those of *Striga* in decreasing root:shoot ratio and enhancing the elongation of the stem, but the treated plants were abnormally slender and in the presence of *Striga* there was not the alleviation of weight loss that had been hoped for. Chlormequat, conversely, enhanced the effects of *Striga* in reducing stem length and increasing root:shoot ratio. *Striga* emergence in this experiment was not significantly affected by chlormequat or GA treatments but in another test (not reported here), chlormequat caused an acceleration of *Striga* emergence.

Varietal experiment

Partial results of this experiment are presented in Figure 1 and Table 3 to show the relationship between the fresh weight of *Striga* and the corresponding reduction in shoot fresh weight of sorghum. The relationship appears to be a simple linear one with a good correlation of host weight loss, with weight of *Striga*, independantly of species. It may be noted that the weight of individual plants is very much lower in *S. asiatica* than in *S. hermonthica*, and there was no simple correlation at all between *Striga* numbers and loss of host weight.

DISCUSSION

The sequential harvest experiment emphasises the profound way in which *S. hermonthica* can influence the growth of the host, especially at low nitrogen levels. Significant reduction of stem growth, and shoot weight occurs at the very earliest stage of *Striga* attachment when the amount of

Fig. 1 Effects of different Striga samples on shoot weight of 3 sorghum varieties. Striga species, origin and numbers emerged are shown in Table 3 below.

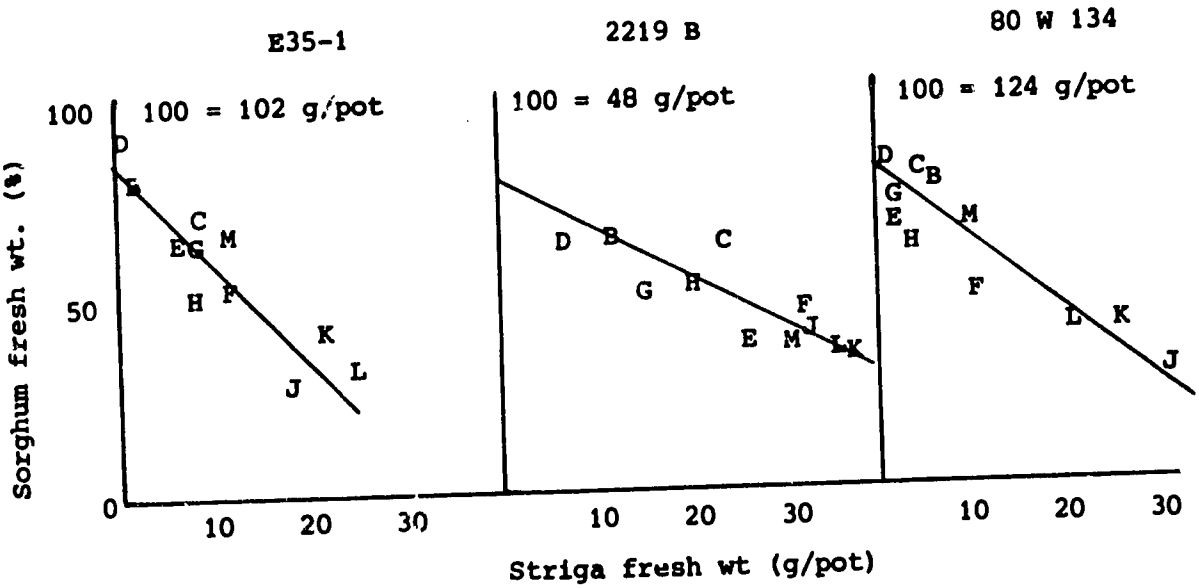

Table 3.

Striga species origin and numbers emerged on 3 sorghum varieties

	Striga species	Origin	WRO No	No. emerged per pot on variety: E35-1	2219B	80W134
B	S. asiatica	India	79-4	40	16	24
C	S. asiatica	S. Africa*	75-1	95	50	24
D	S. densiflora	India	76-1	12	2	4
E	S. hermonthica	Nigeria	77-38	15	25	26
F	S. hermonthica	Upper Volta	77-39	37	14	20
G	S. hermonthica	Gambia	79-4	33	15	19
H	S. hermonthica	Nigeria	77-19	26	17	31
J	S. hermonthica	Niger	79-1	48	35	26
K	S. hermonthica	Sudan	75-1	32	23	21
L	S. hermonthica	Sudan	79-8	25	16	16
M	S. hermonthica	Ethiopia	78-4	23	13	17

* host maize - all others from sorghum host

material removed from the host is relatively quite insignificant. At 4 weeks, less than 1 mg of *Striga* resulted in a reduction of 400 mg in the total weight of the host, and at 5 weeks 13.5 mg caused a total weight loss of 950 mg in the host. There is clearly some very powerful toxin or growth regulator either released from, or induced by the parasite. That it is not a simple toxin, however, is apparent from the lack of toxic effect on the roots which are significantly increased as a result of *Striga* infection. It had been hoped the experiment would confirm whether the stimulation of roots so often seen in previous experiments, preceded or followed the effects on the shoot. If it only followed the stunting of shoots it could be explained as an indirect consequence of the suppression of shoot growth and the passive re-direction of photosynthate to the root system. At low N this was the case, but at high N increase in root weight was almost simultaneous with the reduction in shoots leaving some question whether there is some element of direct root stimulation, independant of shoot inhibition. It can, however, be noted that there is never any observed stimulation of growth or branching of the individual infected roots and it is the whole root system that benefits from the stimulus. This is borne out by the increase in the numbers of prop roots which result from *Striga* attack. Also the senescence of lower leaves is delayed by *Striga* attack, again suggesting a diversion of surplus food from above rather than a localised sink effect in the roots.

S. asiatica and *S. densiflora* are much smaller plants than *S. hermonthica* and individually have a much less serious damaging effect on sorghum. The data presented here from the varietal experiment, however, suggest that when compared on the basis of *Striga* weight they are comparable and that there is a quite close correlation between *Striga* weight and host weight loss over a wide range of *Striga* intensities.

Since the sequential harvest experiment was conducted, Drennan and El Hiweris (1979) have shown how the inhibition of sorghum shoot growth by *S. hermonthica* can be explained by a dramatic change in the ratios of growth promoters and inhibitors reaching the sorghum shoot in the xylem sap. It is still not confirmed, however, whether this is adequate to explain the stimulation of roots. Nor is there as yet any explanation of how the *Striga* induces the changes in growth substances, whether it is, (i) triggered by a substance diffusing from parasite to host, against the main host-to-parasite flow or, (ii) a form of wound response. Although (i) is not supported by the observations of several workers who found negligible parasite-to-root movement of C_{14} in radio-label experiments (e.g. Rogers & Nelson 1962) it may be noted that *S. hermonthica* causes chlorotic blotch symptoms in its host which are not caused by *S. asiatica*, suggesting the existence of a toxic substance rather than a wound response (which would not be expected to differ between *Striga* species). Whether the initial cause is toxin or wound response, it appears probable that it is closely linked to the response of the plant to drought. Drennan and El Hiweris (1979) noted the parallel between drought and *S. hermonthica* in their effects on growth substance balance in sorghum. It also seems likely to be significant that *S. asiatica* characteristically induces wilting in its host, even under moist soil conditions.

The observation of reduced stem elongation and some earlier results showing enhancement of *Striga* effects by chormequat suggested that the damage might be alleviated by treatment with gibberellin. Several attempts have been made, but the sample of results shown above are typical and not encouraging. Stem length can be restored by a spray of GA but growth is still abnormal and sorghum weight is still seriously reduced.

The effect of nitrogen in the sequential harvest experiment was less pronounced than in some others, especially in the relatively short delay of only one week in *Striga* development. The delay in serious damage to the host was considerably greater however - about 3 weeks - and would no doubt have been more prolonged under conditions (e.g. larger pots, or in the field) where the soil nitrogen would not have been so rapidly depleted.

Although the percentage reduction in sorghum weight due to *Striga* was somewhat less with high nitrogen, the actual reduction in sorghum dry weight per mg of *Striga* weight was not appreciably different. Other experiments have also failed to show any consistent increase in the tolerance of sorghum to *Striga* at high N. The nature of the nitrogen effect remains elusive. Teferedegn (1973) suggested an effect via reduction of stimulant exudation and hence a delay in *Striga* germination but this has not yet been fully confirmed. From the experiment described above, it might be hypothesised that a large part of the nitrogen effect could be via its effect on the partitioning of photosynthates between root (+*Striga*) and shoot. In the absence of *Striga* it significantly reduced root:shoot ratio and in the presence of *Striga* it greatly delayed the increase of root:shoot ratio in infected plants.

Acknowledgements

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A STUDY ON "WET DORMANCY" IN SEEDS OF STRIGA HERMONTHICA (DEL.) BENTH. IN VITRO

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Abstract. Secondary or "wet dormancy", which has been described for seeds of the parasitic weed Striga hermonthica (Del.) Benth., was reinvestigated. When the conditioning period, i.e. the period that the seeds are kept under moist conditions in vitro before exposure to a germination stimulant, was extended, the germination percentage approached zero after 10 to 14 weeks. On the basis of a tetrazolium colour test for seed viability it seems likely that under the influence of a prolonged conditioning period the seeds enter a state of dormancy. However, in contrast to the results of a previous study, the dormancy of the seeds could not consistently be broken by drying and repeating the conditioning. The role of "wet dormancy" under natural conditions is discussed.

INTRODUCTION

In the parasitic weed Striga hermonthica (Del.) Benth. seed germination requires very specific conditions. These include a period during which the seeds are kept under moist conditions, generally called conditioning (or pre-treatment), and an impulse by a chemical stimulant (Vallance, 1950; Parker, 1965). The length of the conditioning period has been shown to influence the number of germinating seeds (Vallance, 1950; Reid & Parker, 1979). The germination percentage is generally maximal when conditioning is carried out for approximately two weeks. Vallance (1950) reported that, if this period is extended the germination percentage decreases and may ultimately approach zero. He showed that this is not caused by a loss in viability as the effect was nullified when the seeds were dried and once more conditioned. As a consequence Vallance (1950) concluded that the seeds enter a state of dormancy which was designated by the term "wet dormancy". Reid & Parker (1979) also noted a decrease in germination of S. hermonthica after a prolonged conditioning period but the germination of the seeds was not tested after subsequent drying.

In West Africa the development of S. hermonthica seems to be correlated with rainfall and the length of the crop growing period in relation to the wet season (Ogborn, 1972). When sorghum is sown at the beginning of June when the rainy period starts, the bulk of Striga plants appear above the soil surface immediately after the ending of the heavy rains in September. Ogborn suggested that during a continuous rainy period the seeds enter a state of "wet dormancy". He noted that in Nigeria Striga infestation was especially heavy after a short dry period during the wet season and hypothesized that this was due to breaking of the "wet dormancy". More recently Stoop et al. (1981) also connected "wet dormancy" with the severity of Striga infestations. They observed that in Northern Ghana the weed most fre-

quently occurs on soils which are prone to moisture stress, i.e. soils with a coarse structure and low organic matter content, and suggested that on these soils the "wet dormancy" is more readily broken.

In the present study the occurrence of "wet dormancy" in S. hermonthica was reinvestigated. After various periods of conditioning, whether or not followed by drying and subsequent conditioning, the germination percentage was assessed in the presence of a germination stimulant. In certain experiments the viability of the seeds was also determined by means of a tetrazolium colour test.

METHODS AND MATERIALS

The origin of the seeds of S. hermonthica used in the present study, are presented in Table 1. In the laboratory the seeds were stored in the dark

Table 1 Details of origin of the various seed samples of Striga hermonthica.

Sample no.	country of origin	year of collection
1	Nigeria, Samaru	1977
2	Sudan, Kadogli	1978
3	Sudan, Wad Medani	1978
4	Sudan, Shambat	1974
5	Sudan, Abu Naama	1977
6	Upper Volta, Benena Djib	1981
7	Upper Volta	1980
8	Sudan	1976

at 5°C and a relative humidity of approximately 70% (no data are available on storage between harvesting and arrival of the seeds in the laboratory in Amsterdam). Two synthetic germination stimulants, the strigol analogues GR 7 and GR 24, were supplied by Professor A.W. Johnson of the University of Sussex, Brighton, UK. Two groups of experiments were carried out. Each group included experiments on the effect of the length of the conditioning period on germination (to assess whether the germination percentage decreases, i.e. whether "wet dormancy" is induced, after a prolonged conditioning period), and, following a prolonged conditioning period, the effect of drying and a second conditioning period on germination (to assess whether a decrease in the germination percentage, i.e. the supposed "wet dormancy" phenomenon, is nullified by drying). An overview of the two groups of experiments is presented in Table 2.

Table 2 An overview of the two groups of experiments.

Group of experiments	Length of the conditioning period (weeks)	Length of the period that the seeds were kept dry following prolonged conditioning (weeks)	Second conditioning period (weeks)
I	0 - 10	1 - 33	2
II	0 - 12 or 14		0 - 7

The experiments in Group I were conducted in 1981, the experiments in Group II in 1982. For practical reasons there were minor differences between the methods which were applied in each group.

The experiments were carried out in the dark on glass fibre filter paper (to reduce fungal problems) in 9 cm diameter Petri dishes. In Group II, where the seed samples appeared to be highly contaminated, the seeds were sterilized with a 2% NaOCl solution for 5 minutes.

For conditioning the seeds were placed on moist 1 cm diameter discs of Whatman GF/A glass fiber filter paper (20-30 seeds per disc) and subsequently the discs were arranged on two layers of Whatman GF/A glass fiber filter paper (diameter 9 cm) wetted with 8 ml demineralized water. For each treatment there was one Petri dish in Group I and there were five Petri dishes in Group II. Each Petri dish contained five discs with seeds. In Group I there were three replicates. The Petri dishes were placed in an environmental chamber at a temperature of 25°C ± 2°C. After the conditioning period the discs were taken from the Petri dishes and dabbed on dry filter paper to remove surplus moisture. Subsequently, for the germination test the 1 cm discs were placed in new Petri dishes: in Group I on rings of filter paper (wetted with demineralized water) 0.5-1 cm wide which were cut from 9 cm diameter Whatman No. 1 filter papers, in Group II on glass fibre filter paper discs with a diameter of 2 cm (cut from Whatman GF/A paper): The 1 cm diameter discs in Group I were moistened with 0.02 ml of GR 24 solution, in Group II with 0.2 ml GR 7 solution (the concentration of the GR solutions was 1 mg l^{-1}) or (in separate Petri dishes) 0.2 ml of a 0.6% solution of triphenyl tetrazolium chloride. The tetrazolium colour test (TTC test) is a means for determining seed viability (Moore, 1973). The 2 cm discs in Group II were used in order to maintain the moisture content of the 1 cm diameter discs for a longer period of time than on the rings which were applied in Group I. In Group I the exposure to the germination stimulant was performed in an environmental chamber at 28°C for 48 hr. In Group II the exposure to the germination stimulant was carried out in an environmental chamber at a temperature of 33°C for 24 hr. The TTC test was conducted for a period of 5 days (at 33°C). In accordance with Moore (1973) the percentage coloured seeds was designated as potential germination percentage.

The solution for the TTC test was prepared by dissolving 6 grams of 2,3, 5 triphenyl tetrazolium chloride in 1 l of a 0.025 M phosphate buffer solution which was kept at a pH of 6.9.

Drying of the seeds (after a prolonged conditioning period) was carried out:
In Group I for 7 days at 25°C and a RH of 30-50%.
In Group II: a) abruptly, over 96% sulphuric acid (at room temperature) for 48 hr, in accordance with Vallance (1950), followed by 21 days in an incubator at 35°C and RH of 30-40%.
b) 23 days in the incubator mentioned under a).

Germination and colouring of the seeds was assessed with a low power microscope.

RESULTS

The effect of the length of the conditioning period on germination is shown in Fig. 1a-5a (Group I) and Fig. 6a-8a (Group II). In general the percentage germinating seeds increased markedly when the conditioning period was extended from zero to two weeks. Subsequently the percentage remained more or less constant and after a period varying from 5 weeks (in Group I) to 7-8 weeks (in Group II) decreased and ultimately (after 10-14 weeks) approached zero. The maximum

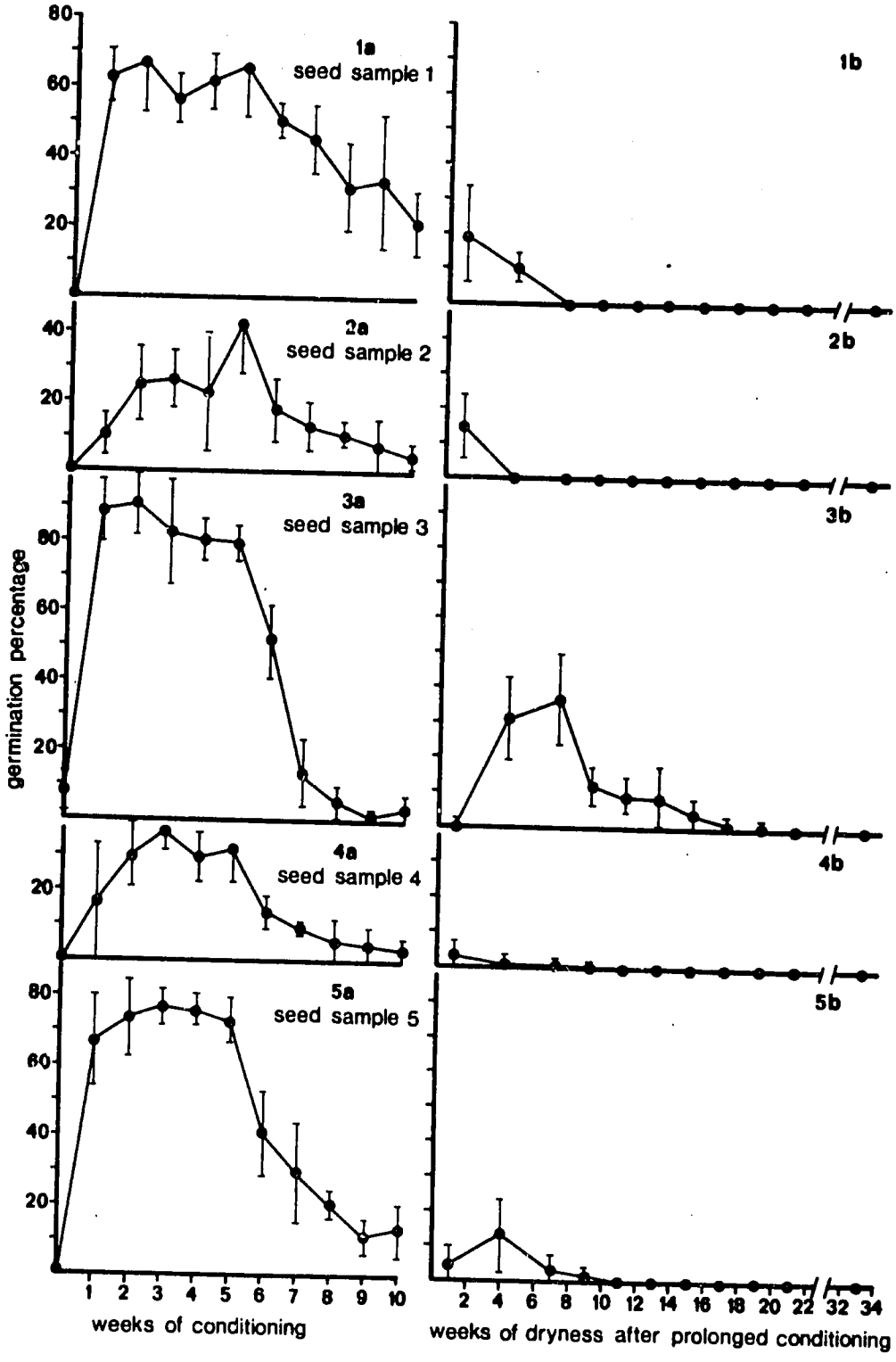

Fig. 1-5. % germination of Striga hermonthica seeds (Group I). a- after various periods of conditioning. b- after a second conditioning period of two weeks following a prolonged (10 weeks) initial conditioning period and various periods of dryness. Vertical bars above and below the mean indicate $t_{0.05} \cdot (s/\sqrt{n})$.

number of germinating seeds varied considerably per seed sample.

The number of seeds which could be coloured with the TTC test (potential germination) was not markedly affected by the length of the conditioning period (Fig. 6a-8a). In seed samples 6 and 7 the maximum germination was in the same range as the potential germination percentage. On the other hand, in seed sample 8 it was markedly lower than the potential germination.

If, in addition to a prolonged conditioning period (10 weeks in seed samples 1-5, 12 weeks in sample 8 and 14 weeks in samples 6 and 7), the seeds were dried and again conditioned, the germination percentage still remained low in samples, other than samples 3 and 8. However, only in sample 8 did the number of germinating seeds reach a level comparable to the maximum obtained after a first conditioning period.

The potential germination percentage also remained more or less constant after a second conditioning period of various length (Fig. 6b-8b). It should be noted, however, that in seed sample 7 the potential germination percentage was considerably lower after the second conditioning period (following drying) than after the first.

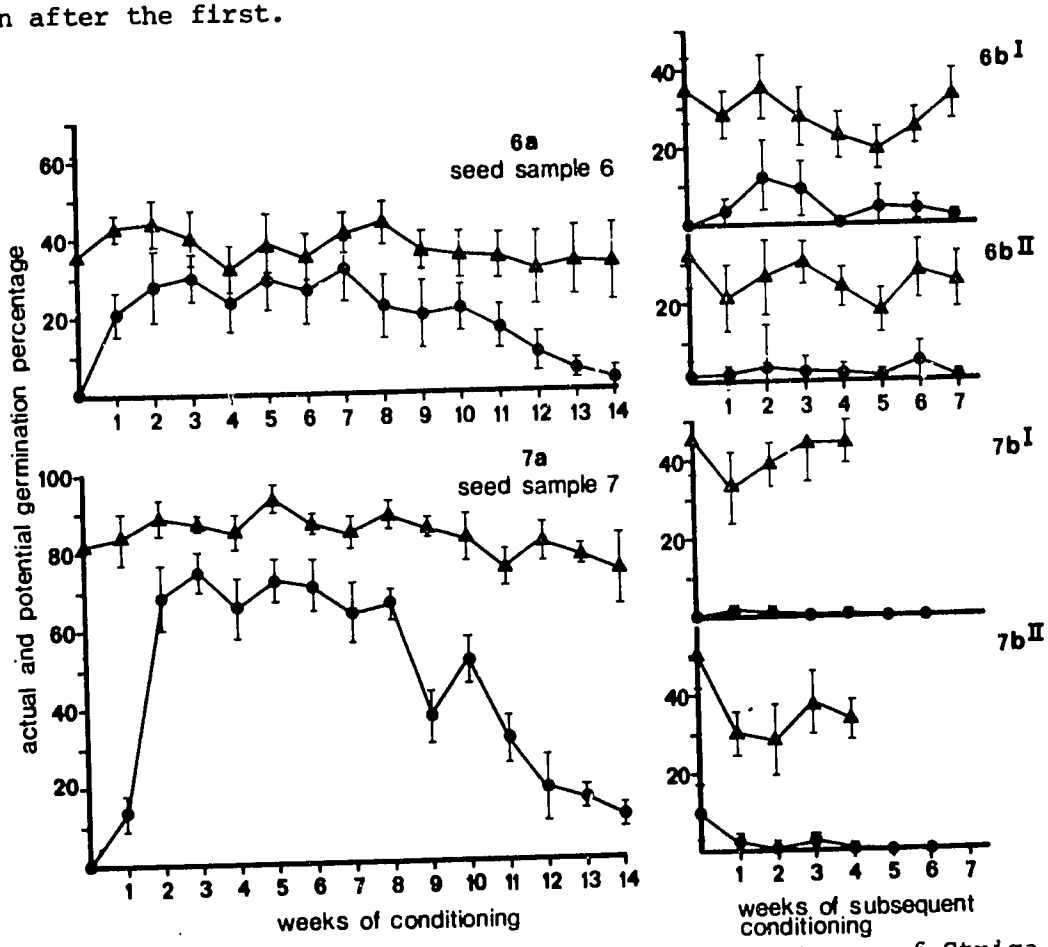

Fig. 6-7. Actual (●) and potential (▲) germination percentages of Striga hermonthica seeds (Group II). a- after various periods of conditioning. b- after various second conditioning periods following a prolonged initial conditioning period (14 weeks), and a dry period of 23 days. The seeds were dried (I) at 33°C in an incubator or (II) abruptly over sulphuric acid at room temperature during 2 days followed by 21 days at 33°C in an incubator. Vertical bars above and below the mean indicate $t_{0.05} \cdot (s/\sqrt{n})$.

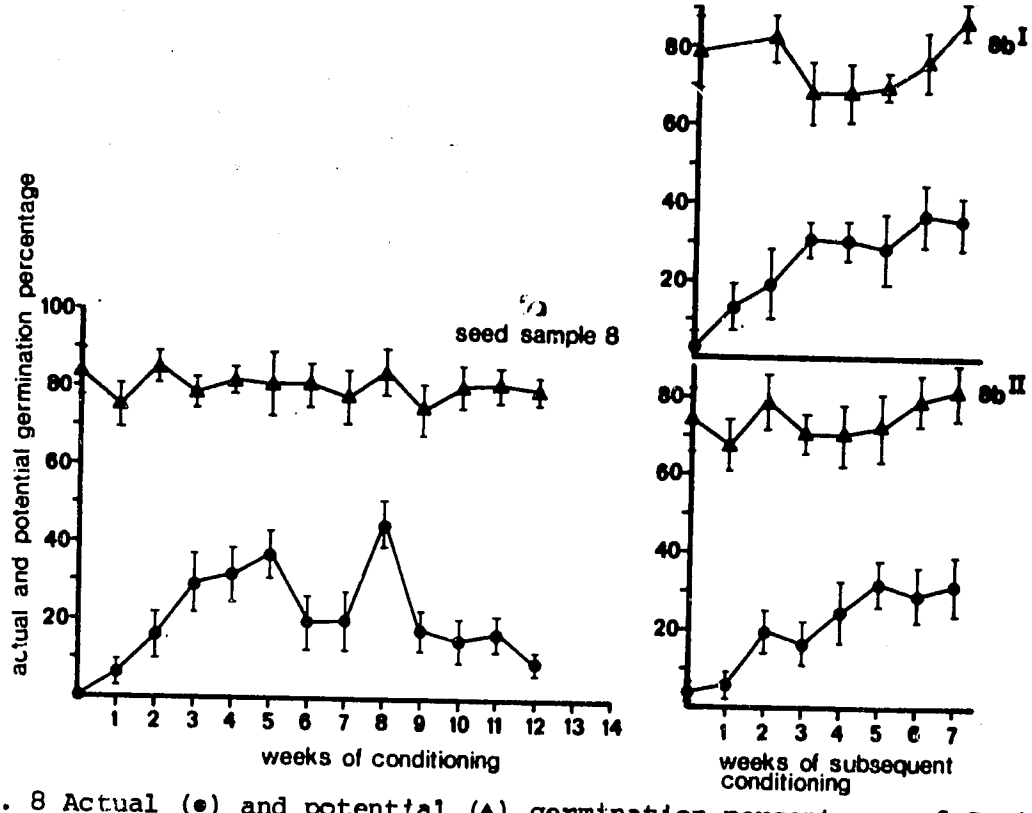

Fig. 8 Actual (•) and potential (▲) germination percentages of Striga hermonthica seeds (Group II). a. after various periods of conditioning. b. after various second conditioning periods following a prolonged initial conditioning period (12 weeks), and a dry period of 23 days. The seeds were dried (I) at 33°C in an incubator or (II) abruptly over sulphuric acid at room temperature during 2 days followed by 21 days at 33°C in an incubator. Vertical bars above and below the mean indicate $t_{0.05} \cdot (s/\sqrt{n})$.

There were no marked differences in germination between the seeds which had been dried abruptly over sulphuric acid and those which had been dried in an incubator (Fig. 6bI/6bII, 7bI/7bII, 8bI/8bII).

When the second conditioning period was varied (Group II: Fig. 6b-8b) the germination percentage increased in seed sample 8 and the maximal level was reached after 3-4 weeks. In seed samples 6 and 7 the length of the second conditioning period did not appear to have a marked influence.

After a prolonged drying period (Group I) the germination percentage became zero (Fig. 1b-5b).

DISCUSSION

The decrease in the percentage germinating seeds after a prolonged conditioning period, which had been reported previously by Vallance (1950) and Reid & Parker (1979), was also observed in the present investigation. The period which was required for the germination percentage to approach zero (10 to 14 weeks) was much longer than the period (3 to 4 weeks) reported by Vallance (1950). That the decrease in germination was not always apparent in the experiments by Reid & Parker (1979) could be due to the fact

that the seed were not exposed to a conditioning period longer than 8 weeks. It is not clear why in the experiments by Vallance (1950) the inhibiting effect on germination was brought about after such a short period. Physiological and genetical differences among the seed samples could play a role.

The assumption of Vallance (1950) that after prolonged conditioning the seeds enter a state of secondary dormancy, i.e. "wet dormancy", rather than losing viability seems to be confirmed by the TTC test, as the number of seeds which could be coloured remained more or less constant. However, in contrast to Vallance's work the germination of "wet dormant" seeds, with the exception of sample 8 and, to a lesser degree sample 3, did not increase after drying and subsequent conditioning. Hence, it appears, that in most cases "wet dormancy" is not readily broken by drying of the seeds.

It seems unlikely that the divergence from Vallance's results is due to the method by which the seeds were dried. In the present study the same results were obtained when the seeds were dried abruptly over sulphuric acid, in accordance with the method described by Vallance (1950), or when the seeds were dried in an incubator.

With regard to sample 8 it is striking that in contrast to the other samples which were exposed to the TTC test (samples 6 and 7) there is a relatively large difference between the potential germination percentage and the maximal germination percentage (see Fig. 8a and Fig. 6a/7a). As a consequence a large amount of the viable seeds did not germinate. This implies that the relatively large number of seeds of sample 8 which germinated after the second conditioning period (Fig. 8b), have not necessarily passed through a state of "wet dormancy". It is conceivable that these seeds belonged to the viable seeds which did not germinate after the first conditioning period.

As far as the TTC test is concerned it should be noted that this test has also been used for determining the viability of seeds of S. asiatica (Kust, 1963). There was no appreciable decrease in colouring capacity of the seeds during a 15 week test at 20% RH and 31°C. However, when these seeds were stored at 100% RH and a temperature of 31°C they could not be coloured 11 weeks after storage began. This suggests that the seeds of S. asiatica lose their viability when they are stored under moist conditions instead of entering a state of "wet dormancy". Reid & Parker (1979), on the other hand, reported that after a conditioning period of 8 weeks the decrease in the germination of S. asiatica was less pronounced than that of S. hermonthica seeds.

The hypothesis that "wet dormancy" plays an important role under field conditions, i.e. during the rainy period (Ogborn, 1972), is not corroborated by the results of the present study. Apart from the fact that the "wet dormancy" could only sporadically be broken by drying, there are other complications in connection with the hypothesis of Ogborn. The development of the parasite below the surface will take several weeks (Saunders, 1933; Doughty, 1942; Andrews, 1945). If the plants emerge at the end of September the germination must have taken place during the rainy period, i.e. under conditions when it is very unlikely that the "wet dormancy" can be broken by drying.

In general it has been shown that a high soil moisture is unfavourable for Striga development. For example in the Gezira in the Sudan it has been reported that lightly irrigated soils were more severely infested with S. hermonthica than heavily irrigated soils (Andrews, 1945; Wilson Jones,

1953). It is conceivable that apart from "wet dormancy" in soaked soils also the oxygen/carbon dioxide balance plays a role. It has been shown that seed respiration is markedly enhanced if the seeds, following an optimal conditioning period, are exposed to a germination stimulant (Vallance, 1951, a, b). Therefore in soaked soils oxygen depletion might inhibit germination. With our present knowledge it is not possible to speculate upon the hypothesis of Reid & Parker (1979) that the rate at which "wet dormancy" develops in the field is influenced by the oxygen/carbon dioxide balance. In the laboratory "wet dormancy" is induced when the seeds are imbibed on the surface of wet filter paper in open contact with the air. Another influence of excessive moisture which could be considered to affect germination in the field is that the effectiveness of the germination stimulant might decrease because of a dilution of the root exudate of host plants. It seems unlikely that the stimulatory effect of short drying periods on Striga emergence, as reported by Ogborn (1972), can be solely attributed to a breaking of "wet dormancy". With regard to the severity of the problems with S. hermonthica, especially in the Sahel region in northern Africa where the weed to a large extent reduces the production of the staple foods sorghum, millet, maize and rice (Pieterse and Daams, 1980; Ramaiah, 1981; Stoop et al., 1983), further research on the interaction between seed germination and soil moisture is strongly recommended.

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STUDIES ON THE GERMINATION OF SOME INDIAN COLLECTIONS OF STRIGA ASIATICA (L.) KUNTZE

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Abstract. From field observations and laboratory studies on the seed germination of samples of the root parasite - *Striga asiatica* (L.) Kuntze collected in Karnataka, India, it is suggested that these samples could be distinct intraspecific variants. Study of these samples with regard to their after-ripening, the duration and temperature of pretreatment, their temperature sensitivity during germination and their response to ethephon, L-methionine, cyclic adenosine monophosphate during germination also confirm their physiological distinctness. These variants are adapted to different host crops and to the climate of the places of their collection. Further data on a sample infesting *Paspalum scrobiculatum* L. confirms that it differs from forms attacking sorghum and millet in its germination behaviour, phenolic content and proteins. The significance of these findings is discussed.

INTRODUCTION

The field observations and studies on the germination of different collections of *Striga asiatica* (L.) Kuntze from the fields of sorghum [*Sorghum bicolor* (L.) Moench] and millet (*Pennisetum typhoides* Rich.) in different parts of Karnataka State, India led to the suggestion that these could be distinct intraspecific variants (Bharathalakshmi and Jayachandra, 1979). After-ripening and pretreatment are important constituent phases in the germination of the parasite; hence the studies were extended to examine if these Indian collections of *S. asiatica* varied with regard to these two phases and their temperature sensitivity during germination. Their response to ethephon and L-methionine that are reported to induce germination in the parasite (Egley and Dale, 1970; Worsham, 1961) and cyclic adenosine monophosphate (cAMP) in the germination medium has also been tested. Further studies are also reported for the sample from *Paspalum scrobiculatum* L. for comparison with those already published for samples from sorghum and millet (Bharathalakshmi and Jayachandra, 1979).

METHODS AND MATERIALS

Three different samples of *Striga*, two from sorghum fields

at Kikkeri (Mandya district) and H.B. Halli (Bellary district), and one from Paspalum fields at Kikkeri have been considered for this study; the former two being designated as SK and SH, and the latter as PK. Temperatures at Bellary are generally 3-5°C higher than those at Kikkeri. The rainfall at these two places of collection has a common pattern of distribution with a minimum of 30 mm in June rising steadily by September to about 100 mm/month at Bellary and 125 mm/month at Kikkeri.

Sorghum used throughout the study was cv. CSH-1. Local var. of Paspalum was obtained from cultivators at Kikkeri.

The Striga seeds were after-ripened for atleast six months (unless otherwise stated), pretreated and germinated as described earlier (Bharathalakshmi and Jayachandra, 1979).

1. After-ripening

The SK, SH and PK samples of Striga were subjected to after-ripening for 1-12 months by storing them in plastic containers under laboratory conditions of temperature (23-35°C) and relative humidity (35% in March-April to 98% in September). Subsequently, the seeds were pretreated at 35°C for 10 days and tested for their germination response to the root exudate of the respective host in five replications of four discs each.

2. Pretreatment temperature and duration

The seed samples of SK, SH and PK were pretreated at 20, 25, 30, 35 and 40°C for 10 days in a 'Biochem' BOD incubator and germinated in GR 7 (1 µg/ml) medium with six discs in two sets. GR 7 originated from the university of Sussex, UK (Johnson et al., 1976). Another set pretreated for 0 - 20 days at 35°C was also germinated in the root exudate of the respective hosts using five replications of four discs each.

3. Germination at different temperatures

The three samples of Striga seeds pretreated for ten days at 35°C were tested for germination at 20, 25, 30, 35 and 40°C in GR 7 (1 µg/ml) medium with six discs for each treatment.

4. Response to synthetic chemicals in the germination medium

The after-ripened and pretreated Striga seed samples were set for germination in solutions of ethephon (commercial formulation - 1, 10 and 100 µg/ml), L-methionine (10 and 100 µg/ml) and cAMP (1 and 10 µg/ml) in three replications of four discs each.

5. The PK sample of Striga seeds were tested for germination in root exudate and on root system of sorghum, pearl millet and

Paspalum as described earlier (Bherathalakshmi and Jayachandra, 1979).

6. The seeds of PK, pretreated for 0, 5 and 10 days at 35°C were set for germination in four replications of four discs each in 0, 10, 25, 50, 75 and 100 µg/ml of kinetin.

7. The 10 day-pretreated PK seeds were also germinated in 1, 10 and 100 µg/ml of GR 7 in five replications of four discs each. The germination was recorded at 24 h.

8. The total phenolic level in the seeds of PK sample of Striga was determined following the method described in A.O.A.C. (1960).

9. The aqueous extract of the PK seeds (100 mg/ml) was subjected to polyacrylamide gel electrophoresis following the procedure described by Plummer (1979). The protein bands were stained with amido black and their intensity was read at 640 nm in a 'Biochem' densitometer.

RESULTS

Fig.1 shows that in the first two months after harvest, only SK germinated. SH and PK began germinating only after 2-3 months storage. All samples then gave increasing germination to a peak between 6 and 8 months followed by a marked decline through 12 months.

SK and PK each showed some germination with 4 day pretreatment while SH began to germinate only after 12 days pretreatment (Fig.2). Germination of SK was maximum after 12 days while SH and PK showed maxima at 16 days. All showed a moderate decline after their peak.

The samples were responsive to pretreatment temperature over the range of 25 to 35°C, all showing maximum germination at 35°C and nil at 20° or 40°C (Fig.3).

The three samples of Striga germinated to different degrees, over the range of 25 to 35°C (Fig.4). SK and SH both showed maximum germination at 35°C, while PK germinated best of all at 30°C.

Only SK and SH samples germinated in ethephon and only SK in cAMP or methionine (Fig.5).

PK was germinated in root exudate/root system of only P. scrobiculatum and not by those of sorghum or millet. The per cent germination recorded were 33 in root exudate and 23 on root system.

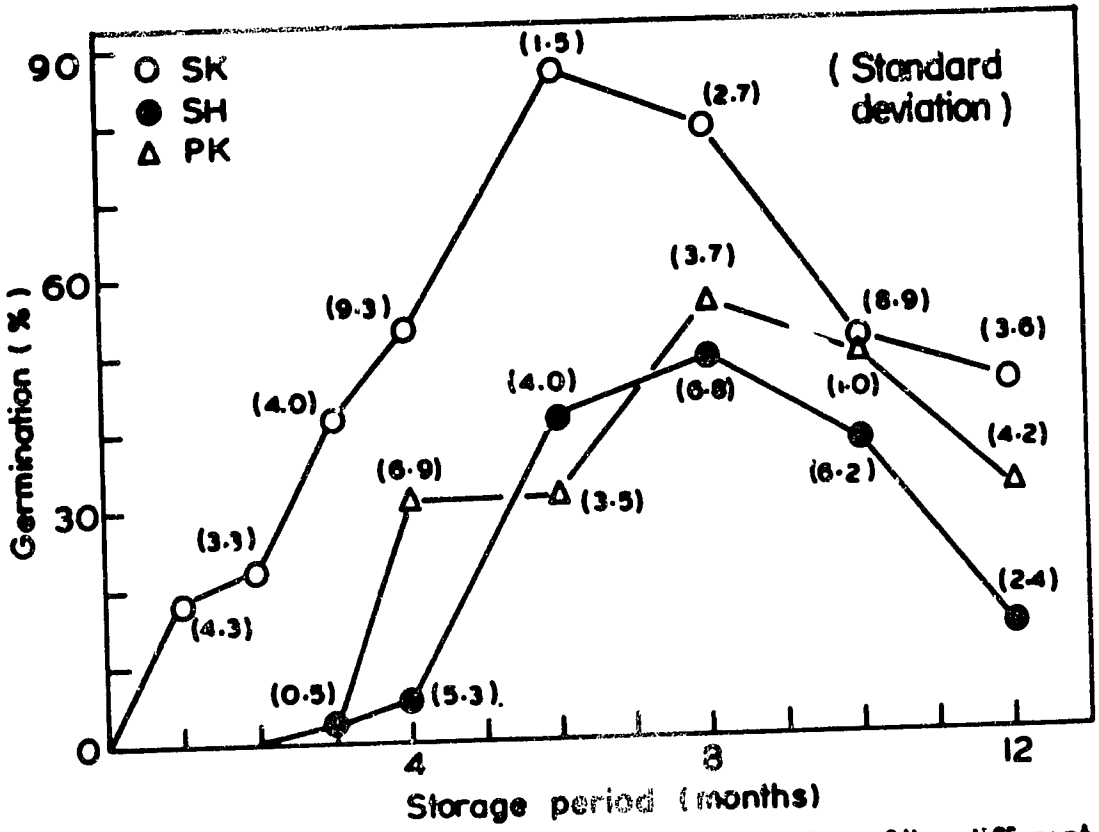

Fig. 1. The effect of storage on the germination of the different field samples of S. asiatica in the root exudate of the respective hosts.

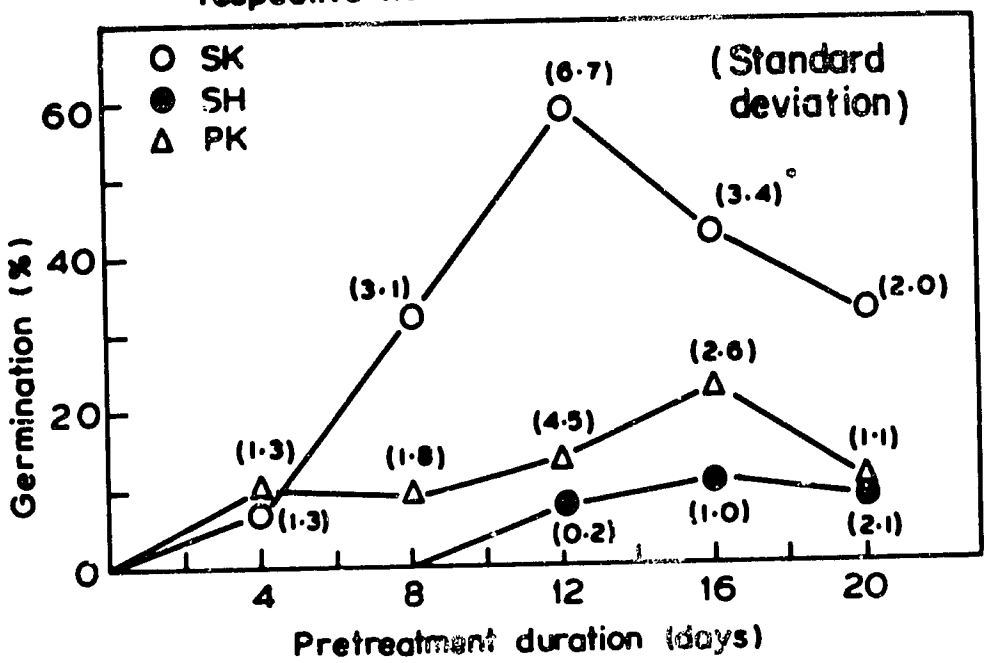

Fig. 2. Effect of the duration of pretreatment on seed germination in the different field samples of S. asiatica

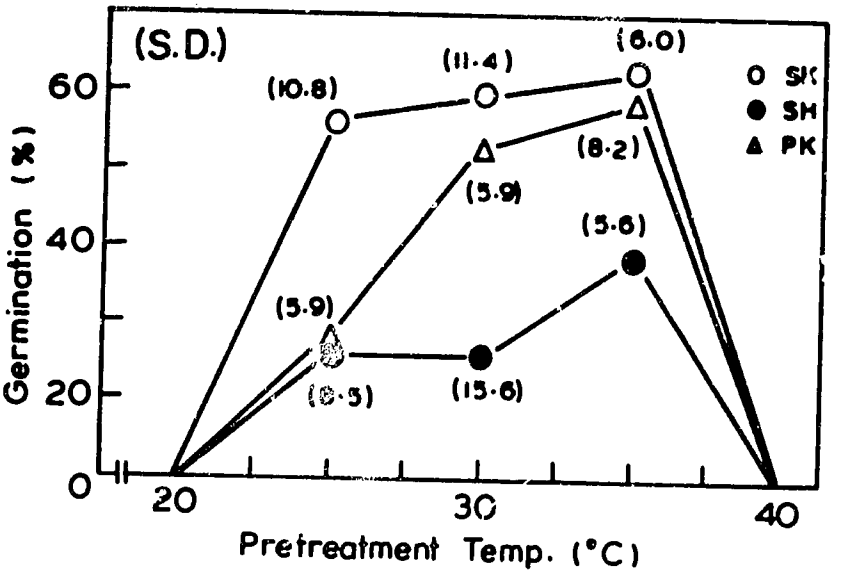

Fig. 3. The influence of pretreatment-temperature on seed germination in the different field samples of S. asiatica in GR 7 (1 μg ml⁻¹) medium.

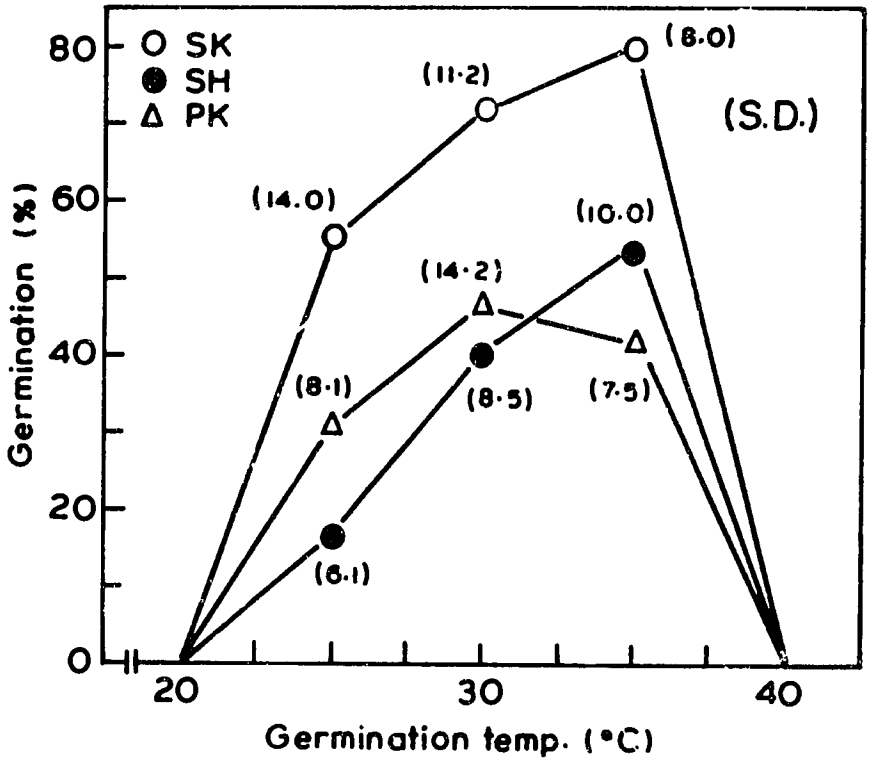

Fig. 4. Seed germination in GR 7 (1 μg ml⁻¹) of the different field samples of S. asiatica at different temperatures following pretreatment at 35°C for 10 days.

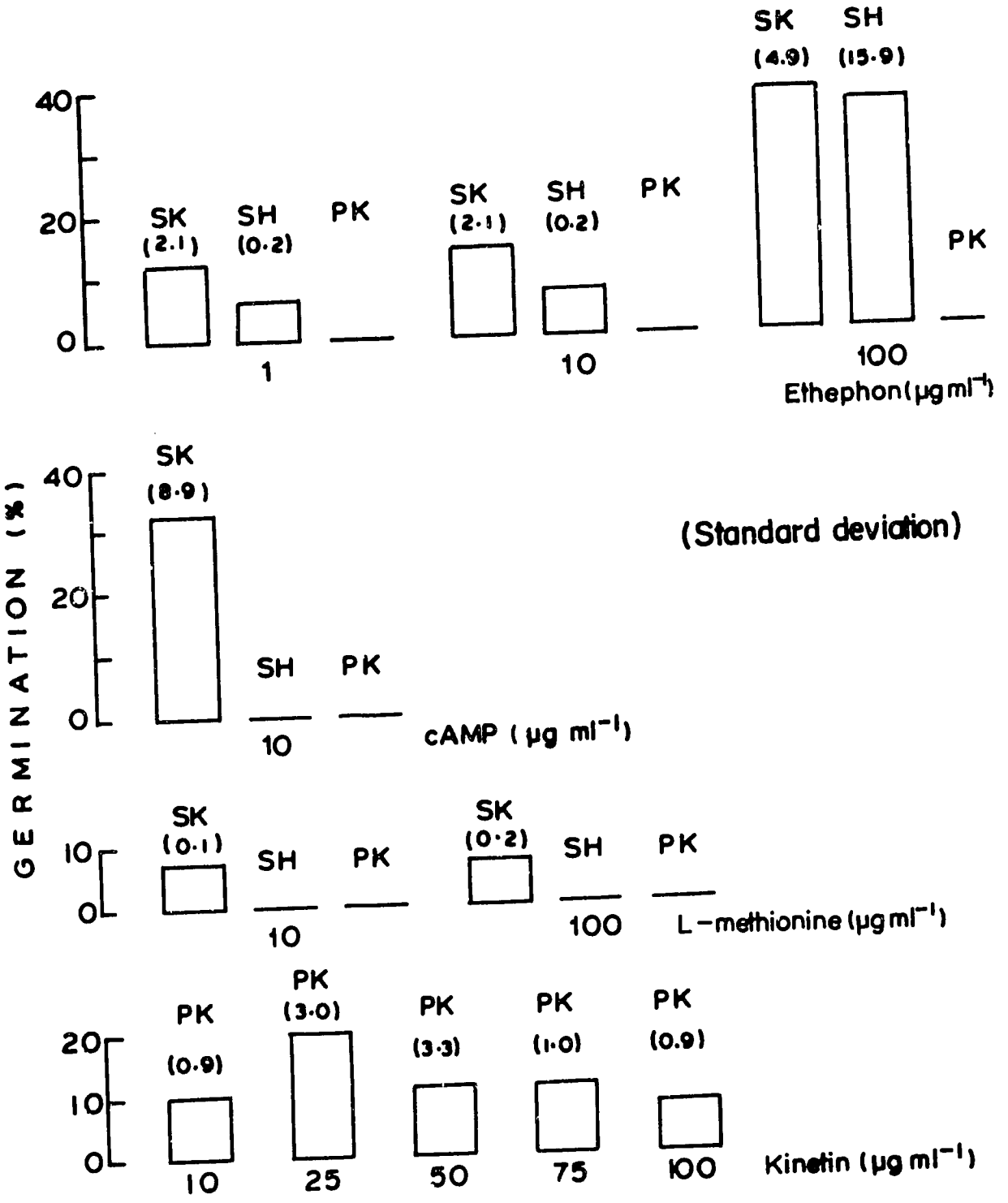

Fig. 5. Seed germination of the different field samples of S. asiatica in the media of different synthetic compounds.

The sample PK could respond to kinetin only if pretreated; with five day pretreatment the germination occurred only at 25 (9%) and 50 µg/ml (4%). With the extension of pretreatment to 10 days, germination occurred at all the concentrations tested (Fig.5).

PK seeds gave 55% germination at 1 µg/ml of GR 7; at 10 µg/ml, it was reduced to 13%. Further increase in the concentration to 100 µg/ml, the germination was inhibited totally.

The total phenolic level was 425 µg/g of PK seeds as compared to 380 µg/g in SK and 530 µg/g in SH (Bharathalakshmi and Jayachandra, 1979).

The electrophoretic separation of seed proteins showed seven distinctly visible bands (Fig.6).

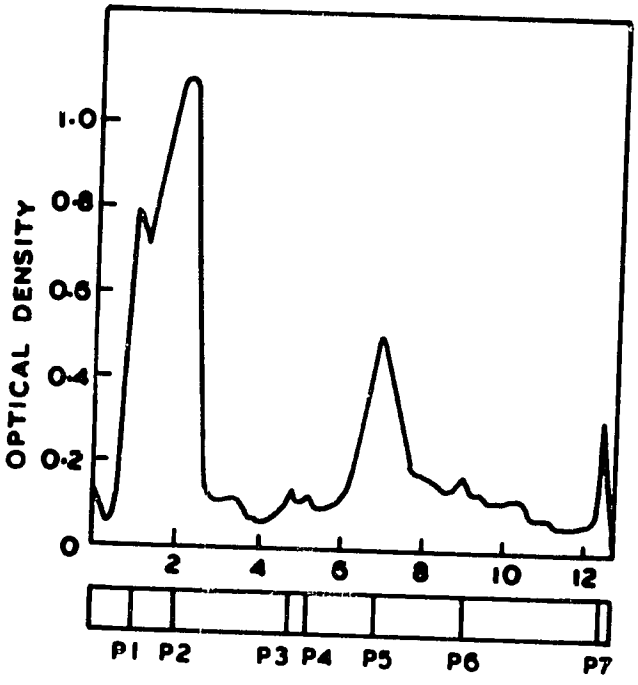

Fig. 6. The electrophoetic pattern of seed proteins in PK

DISCUSSION

The data in Fig.1 show that the three samples of Striga varied in their requirement of after-ripening. SK that after-ripened faster than SH and PK showed considerable germination with storage for just one month and this was comparable to the Abu Naama strain of S. hermonthica that gave remarkably high

germination within three months of storage (Reid and Parker, 1979). The germination in all the samples declined with storage beyond 6-8 months and similar adverse effects of prolonged storage on viability has been reported by Kust (1963).

The Striga samples of the present study differed significantly in their response to pretreatment duration. SK and PK responded to four days of pretreatment whereas in SH a comparable percentage of germination occurred only with 12 days of the treatment. The decline in per cent germination of the SK and PK samples under prolonged pretreatment is in conformity with the reported adverse effects of similar treatment in the non-Indian strains of S. asiatica (Brown and Ewards, 1944).

Inhibitors from the seeds of Striga are reported to get leached out during pretreatment (Kust, 1966; Hsiao et al.,1979). The seeds of the PK sample of Striga is shown to contain phenolic acids as do those of SK and SH (Bharathalakshmi and Jayachandra, 1979). Phenolics are well known germination inhibitors (Mayer and Poljakoff-Mayber, 1978). SH ranked highest in the total phenolic level and was followed by PK and SH samples. Kikkeri, from where SK was collected receives relatively higher rainfall particularly during the natural pretreatment period of the sample than H.B. Halli. It is proposed that the longer pretreatment required by SH could be due to the higher phenolic content. Likewise, the higher phenolic content of PK than that of SK might have been one of the reasons explaining its longer optimal period than that of the latter though both the collections were made from the same locality.

The samples of Striga differed significantly in their response to the different temperatures during pretreatment and germination. The favourable range of temperature was broader in SK (25-35°C) than in the other two samples. All the three samples differed from the American sample of S. asiatica which is reported to germinate even at 20°C and above 40°C (Nelson, 1957).

The marked increase in germination per cent in SH and PK when pretreated at 35 and 30°C, respectively correlated with the temperatures prevailing at their respective regions of collection. The optimum temperature for germination in these two Striga samples again correlated with the temperatures at Bellary and Kikkeri. Thus the differential response of these samples would reflect their adaptation to the temperature prevailing during the usual pretreatment and germination periods of the parasite at the two different places.

Only Sorghum-Strigas were responsive to ethrel; PK stood unique in not responding to ethrel, a formulation that is found to be very effective as an inducer of seed germination in many weeds including Striga (Chancellor et al., 1971). The sample

SK was distinct in responding to cAMP and L-methionine.

PK sample could germinate with the root exudate/root system of only that crop but not with those of the other host crops pointing to the host-specificity of the sample and thus it differed from the other strains of Striga reported earlier from India (Bharathalakshmi and Jayachandra, 1979). This sample resembled SK in not responding to kinetin without pre-treatment; but the optimal concentration of kinetin and the per cent germination of the pretreated seeds of the former were lower than those of the latter. Paspalum-Striga could tolerate GR 7 at 10 μg/ml in the germination media to a greater degree than the Sorghum-Striga could.

The seed phenolics in PK correlated well with its germination in root exudate/kinetin/GR 7 in comparison with the two strains of Sorghum-Strigas (Bharathalakshmi and Jayachandra, 1979).

The electrophoretic pattern of the seed proteins of Paspalum-Striga was also different from that of Sorghum-Striga. The intensities of P1 and P2 corresponding to A1 and A2 bands of the Sorghum-Striga, were more intense but the optical density of P5, the second major peak corresponding to A3, was lower. The bands P3, P4 and P7 were characteristic of the sample.

Thus the present study reinforces the suggestion made earlier (Bharathalakshmi and Jayachandra, 1979) that these samples are distinct intraspecific physiological variants. This study also points strongly to the occurrence of yet another variant of S. asiatica adapted specifically to the host - Paspalum in India. Paspalum, reported to be singularly free from pests and diseases and also remarkably drought-resistant (Aiyer, 1966), is cultivated in gravelly and poor soils of arid belts and adaptation of Striga to such hardy crops lessens intraspecific competition of the parasite, increases the chances of its survival and thus contributes to its success. Differences among the host crops of the different Striga samples and the weather conditions prevailing at the places of their collection seem to be important in influencing the adaptations in these variants.

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HAUSTORIAL INITIATING ACTIVITY OF SEVERAL SIMPLE PHENOLIC COMPOUNDS.

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Abstract. The ability of several simple phenolic compounds to induce primary haustoria of *Striga hermonthica* (Del.) Benth. has been tested. Activity was shown when a methoxyl group occupied a meta-position and a hydroxyl group a para-position. The most active compound tested was the benzoic acid derivative syringic acid.

INTRODUCTION

Striga species are root parasites and obtain most of their resources from their hosts. Transfer of host resources occurs via haustoria.

Haustoria are thought to be initiated by chemical recognition factors produced by the host roots. Steffens *et al* (1982) have shown that for *Agalinis purpurea* (L) Raf. a high degree of molecular specificity is required for haustorial induction. For the two flavonoid haustorial inducers, xenognosin A and B two major structural features are important:

(i) A meta relationship of hydroxyl and methoxyl groups,
(ii) An alkyl branching ortho to the methoxyl substituent.

Once a haustorium has been initiated, full contact with the host stele only occurs after any physical or chemical defences that the host possesses are overcome (Saunders 1933, 1942; Maiti and House, 1982).

In plant resistance to fungal pathogens and insect pests, phenolic compounds are known to have an important role. Guenzi and McCalla (1966) identified and quantified five phenolic acids in mature plant residues of Oats (*Avena sativa* L.), Wheat (*Triticum aestivum* L.), *Sorghum bicolor* (L.) Moeuch and Maize (*Zea mays* L.). These compounds were p-coumaric, syringic, vanillic, ferulic and p-hydroxybenzoic acids. This paper reports the effect of these and similar phenolic compounds on the induction of primary haustoria of *Striga hermonthica* (Del.) Benth.

METHODS AND MATERIALS

Bioassays were carried out in a Nunclon Microtest Plate containing an 8 x 12 array of flat bottomed wells (dimensions: top 7 mm, bottom 6.4 mm, depth 11.25 mm). Each tray was supplied with a lid.

A S. hermonthica from a sorghum host, collected in Sudan (Abu Naama, 1980) was preconditioned as by Reid and Parker (1979) and then approximately ten seeds were transferred to each well with 50 µl of root exudate (sorghum cv. feterita) (collected as by Parker et al, 1977). The trays were enclosed in a plastic bag and incubated for 24 hours at 33°C. 50 µl of the test solutions was placed in each well, the trays resealed in a plastic bag and incubated for a further 24 hours. Observations were made at x 40 magnification using a binocular microscope. The compounds tested and the concentrations used are shown in Table 1.

RESULTS AND DISCUSSION

The ability of the phenolic compounds tested to stimulate the formation of primary haustoria of germinated Striga is shown in Tables 1 and 2. From these results it can be seen that for haustorial initiating activity to be shown a hydroxyl and a methoxyl group must be present. This is similar to the Agalinis-xenognosin A system of Steffens et al (1982).

Steffens et al (1982) found that for the Agalinis-xenognosin system the presence of hydroxyl and methoxyl groups was important, activity only being expressed when both groups occupied a meta-position. These results show that for S. hermonthica haustoria are initiated when the hydroxyl group occupies a para-position and the methoxyl group a meta-position. In the case of the benzoic acid derivative syringic acid, it would appear that the presence of a methoxyl group at both meta-positions enhanced activity. No compound was tested which contained a hydroxyl and a methoxyl at the meta-position.

Unlike the Agalinis-xenognosin A system (Steffens et al 1982), there does not seem to be a requirement for an alkyl branching ortho to the methoxyl substituent.

Further work is being carried out to test the toxicity of the haustorial initiating compounds towards sorghum and other hosts and the effect of these compounds on attachment and development of Striga spp., the aim being to test whether this could be a practical control strategy.

Table 1.

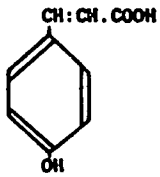
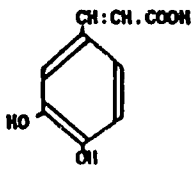
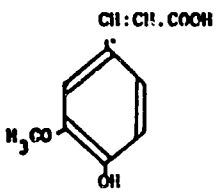
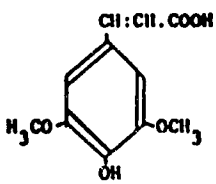
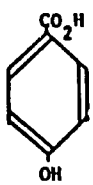
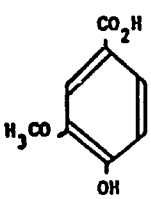
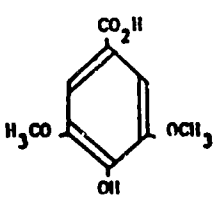
Compounds tested for effects on haustorial initiation in *S. hermonthica*.

Compound	$\bar{x}$ (n=5)% radicles showing haustoria at conc. (mg/l) of test compound							
	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}	10^{2}	10^{3}	CONTROL
p-Coumaric Acid	-	-	-	0(0)*	0(0)	0(0)	0(0)	0(0)
Caffeic Acid	-	-	-	7.4 (7.4)	6.8 (6.4)	10.8(24.1)	2.8 (6.3)	0.65 (2.9)
Ferulic Acid	-	-	-	46.2(28.1)	90.4 (9.1)	33.8(25.6)	0(0)	0(0)
Sinapic Acid	0(0)	0(0)	3.4 (7.6)	8.4(11.4)	87 (12)	83.4(23.5)	0(0)	0.65 (2.9)
p-hydroxybenzoic Acid	-	-	-	5 (11.2)	2.2 (4.9)	0(0)	0(0)	0(0)
Gallic Acid	-	-	-	0(0)	0(0)	0(0)	0(0)	0.7
Vanillic Acid	-	-	-	26.4(34.1)	82 (11.5)	95.6 (6)	0(0)	9.3 (9)
Syringic Acid	6.2 (6.1)	24.8(15.8)	42.8(17.5)	97 (6.7)	98.2 (4)	92.2 (7.2)	15.8(12.2)	9.3 (9)
Vanillin	-	-	7.8(11.4)	34.6 (8.8)	94.2(13)	95 (11.2)	-	0(0)
O-vanillin	-	-	0(0)	0(0)	14.5(17.1)	0(0)	-	0(0)
Scopoletin	-	-	0(0)	0(0)	0(0)	0(0)	-	0(0)
3,5-Dimethoxy Benzoic Acid	-	-	0(0)	0(0)	0(0)	0(0)	-	0(0)

* Figures in brackets are standard deviations. (SD)

Table 2.

Structural formulae of compounds tested, and their effect on haustorial formation in S. hermonthica

Compound		Approximate conc. (mg/l) stimulating 50% of radicles to form haustoria.
p-Coumaric Acid		None
Caffeic Acid		None
Ferulic Acid		1
Sinapic Acid		2.5
p-Hydroxybenzoic Acid		None
Vanillic Acid		2.5
Syringic Acid		0.1

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ASPECTS OF THE RESISTANCE OF SORGHUM VARIETIES TO STRIGA SPECIES

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Abstract In studies on the resistance of sorghum (Sorghum bicolor (L.) Moench) varieties (especially N13) to Striga hermonthica (Del.) Benth., the possibility of three previously unrecognised resistance mechanisms, or contributory factors to resistance, have been identified. Firstly, varieties have been shown to differ in the degree to which they stimulate chemotropic behaviour of Striga seedlings; secondly they may interact differently with soil microflora such that a characteristic rhizosphere flora inhibits Striga development; thirdly varieties may simply differ in root morphology and hence the availability of attachment sites in the upper soil layers. It is proposed that the resistance of N13 may derive from a combination of these factors. There was no evidence that it was influenced by differential haustorial initiation.

INTRODUCTION

During their life cycle, root parasites of the genus Striga require a number of different conditions and stimuli before the seeds are able to germinate, make contact with and attach themselves to a suitable host. Briefly, Striga seeds need a period of warm moist conditions before they are able to germinate (pre-conditioning or pre-treatment), germination can then be triggered by the presence of host root exudates in the vicinity of the seeds. After germinating, the Striga seedling then has to come into contact with a host root, attach itself to that root and penetrate the cortex and endodermis before it is able to tap the host vascular system. The Striga will die if any of these stages in the life cycle fail.

A number of sorghum varieties are known to be resistant to Striga attack for one reason or another, e.g. Framida is believed to be resistant because of low stimulant production (Williams, 1959) yet other varieties, such as N13, are resistant despite high stimulant production (Parker et al, 1977). One purpose of the WRO project is to try and understand these resistance mechanisms, and so help the International Crop Research Institute for the Semi-arid Tropics (ICRISAT) in the development of screening methods (Parker & Reid, 1980). Apart from the generally assumed possibility of mechanical resistance to penetration through endodermal or other tissues (e.g. Maiti & House, 1983), other possibilities include a) that the resistant variety does not elicit the necessary chemotropism in the Striga seedling, b) that it does not elicit haustorial initiation, c) that the rhizosphere microflora of the 'resistant' varieties interferes with Striga development, or d) that the resistant variety simply has less root available for attachment.

This paper describes a number of investigations which have been made, firstly to confirm that Striga seedlings grow towards a source of host

root exudate as reported by Saunders (1933) and Williams (1961) and which is to be expected if the seedlings are to have the greatest chance of survival; secondly to determine if the exudates of resistant and susceptible varieties of sorghum differ in their potential to induce chemotropic responses in Striga.

Once the Striga has contacted a sorghum root, the next stage necessary for successful development is that of haustorial formation. Experiments were set up to compare different varieties of sorghum for their potential in this respect.

The possibility of interaction of soil microflora with Striga was prompted by observations on unhealthy root systems in experiments using the 'polybag' technique (Parker & Dixon, 1983), and experiments were conducted using cultures of organisms isolated from such bags, or natural soil inocula.

The distribution of roots with depth might also have some bearing on the resistance of sorghum to Striga from the simple fact that if a variety of sorghum produces relatively little root in the top 10 cm, then Striga seeds in this zone will have only a slim chance of germinating and successfully contacting a root.

METHODS AND MATERIALS

Chemotropism

The method used initially for these experiments was that used by Whitney and Garraten (1981) for similar studies on Orobanche spp.

Sorghum (Sorghum bicolor (L.) Moench) plants were sown in acid-washed sand, 25 per pot, using the double pot technique (Parker et al, 1977). The 5 cm diameter pots were watered with distilled water and maintained for ten to fourteen days in the glasshouse, as a source of root exudate. Meanwhile, seeds of S.hermonthica, collected from a sorghum host in Nigeria (WRO 77-38), were surface sterilised for 12 minutes in sodium hypochlorite solution (1% available chlorine), rinsed in sterile distilled water, placed for pre-treatment on glass fibre filter paper (Whatman G/FA) in 9 cm petri plates and incubated at 23°C.

Tap water agar plates were prepared using 10 ml of 1.5% agar per 9 cm plate. Small holes (2 mm diameter) were drilled around the base of the plates and a 1 cm diameter circle of agar was removed from the centre to create a well in which the exudate source could be placed (Figure 1).

When pre-treatment of the seed was completed the filter paper was dabbed to remove excess moisture and germination inhibitors, and inverted over the agar. Slight pressure on the filter paper helped to transfer the Striga seeds from the filter paper to the agar plate.

Root exudate in the form of a root solution was removed from the host sand pot by suction. This root solution was then concentrated by

extracting twice with equal volumes of ethyl acetate, evaporated to dryness under reduced pressure, and residues brought back into solution in 10 ml ether. This concentrated stimulant in ether was pipetted onto coils of filter paper (1 cm x 9 cm) and the ether allowed to evaporate. The coils were then placed in the central well in the agar plates, wetted with 1-2 ml of distilled water, and the plates placed in 14 cm dishes with sufficient water in them to cover the holes in the smaller plates thus acting as a sink for the exudate and aiding the development of a concentration gradient of stimulant across the plate. The complete assembly was incubated at 33°C for 48 hours prior to assessment. During this time it was necessary to re-water the stimulant source occasionally.

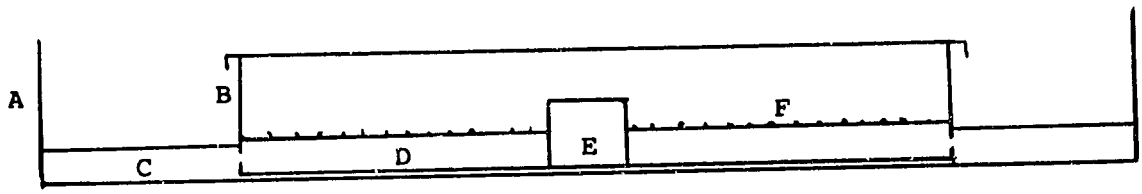

Figure 1. Arrangement for chemotropism study. A. 14 cm petri dish B. 9 cm petri dish C. distilled water D. 1.5% tap water agar E. filter paper coil impregnated with root exudate material F. *Striga* seeds.

Plates were scored by assessing the final direction of the radicle apex; towards (positive), away from (negative), or at right angles to the stimulant source (neutral).

Haustorial initiation

S. hermonthica seeds were sterilised and rinsed as before and then placed onto 8 mm diameter discs of glass fibre filter paper arranged on a full sheet of filter paper in a 9 cm petri plate. The filter paper was watered to a slight excess with distilled water and the plates incubated at 23°C.

A suitable sorghum variety was grown using the double pot technique to provide exudate to germinate the *Striga* seeds, for the first experiment only. Thereafter a 1 mg/l solution of the synthetic germination stimulant GR7 was used (Johnson *et al*, 1976).

Host root material for the first experiment was obtained from 12-15 cm long primary roots of plants cultured in polyethylene bags (Parker and Dixon, 1983) and applied to the discs of *Striga* seeds as 1 cm whole sections. For the second experiment, root material was taken from established plants growing in pots in the glasshouse and applied to discs of *Striga* seeds as 20 µl of a macerate of 1 g of root in 10 ml distilled water, either at the same time as the germination stimulant or 36 hours later. Dishes were returned to the incubator and haustorial initiation was observed 72 hours later. Initiation was assumed to have commenced if the tip of the radicle was swollen and had produced root hairs. Further details are provided in the Results section.

Microflora

These studies all involved the polyethylene bag technique described by Parker & Dixon (1983). Briefly this involves standard 120 gauge polyethylene bags containing a sheet of glass fibre (Whatman GFA) filter paper, and moistened with a suitable nutrient usually 25% Hewitt's solution (Hewitt, 1965). Striga seeds are sprinkled onto the moist paper and the bags are then flattened to exclude air bubbles and suspended by folding the top over a length of split cane. After 7-10 days at 23°C to allow pre-conditioning, a pre-germinated sorghum seed is introduced near the top of the bag. When the sorghum roots are suitably developed, the temperature is raised to 33°C to encourage Striga germination and the progress of germination, attachment and penetration can be followed under a low power microscope.

By suitable sterile techniques the microflora in the bags can be controlled, but considerable care is needed and it was noticed in one 'polybag' experiment that the S. hermonthica seedlings which had not been successful after germinating were heavily infected with bacteria. Samples of these unhealthy Striga were macerated and plated out onto bacterial media (BBR and FP agar media). It was found that those seeds taken from bags containing N13 had a much higher number of bacteria present than those from Feterita. Of these bacteria the majority were identified as pseudomonads. Two distinct types were plated out and cultured for future use.

Three experiments were done to examine the effect of non-sterile conditions on the performance of Striga. The cultured pseudomonads were introduced to the polybags in the nutrient solution or a natural soil microflora was added to the system by dipping pre-germinated sorghum seeds into a slurry of soil and water before introducing them into the bags. The soil was taken either from pots with sorghum (2219B) already established, or from a stock of the same (Yarnton) sandy loam soil.

Root distribution

Observations were made on plants grown using the polyethylene bag technique described above. For the first experiment five seeds each of N13 and Feterita were set up in the polyethylene bags and daily root growth marked on the bag in felt pen. A further two experiments involved the use of an Optimax image analyser to measure root plan area, after careful removal from the bags and filter paper and placement on a black background to give good contrast for the image analyser.

RESULTS AND DISCUSSION

Chemotropism

A preliminary experiment (results not presented) was somewhat inconclusive in that the proportion of Striga seeds showing a tropic response was small. However, amongst those that did show a tropic response, the reaction was significantly different between Feterita and

N13, the tropism being more distinctly positive when Feterita was the source of exudate.

The second experiment in the series confirmed the different response of S. hermonthica to the exudates from N13 and Feterita (Table 1).

Table 1.

Chemotropic behaviour of S. hermonthica in response to root exudates of two sorghum varieties

Sorghum variety	Orientation*		
	Positive	Negative	Neutral
Feterita	489 (45.7)	288 (26.9)	294 (27.4)
N13	244 (35.8)	262 (38.5)	175 (25.7)

* Figures are totals of five replicate plates, figures in brackets are expressed as a percentage of those germinated.

The third experiment in the series extended the number of sorghum varieties examined to four, two resistant (N13 and Framida) and two susceptible (Feterita and 2219B). Data for the first two zones only are presented in Table 2 as beyond zone two the differences were insignificant.

Table 2.

Chemotropic behaviour of S. hermonthica in response to four sorghum varieties.

Sorghum variety	Orientation			
	Zone 1 (0 - 1.5 cm from source)		Zone 2 (1.5 - 2.5 cm from source)	
	positive	negative	positive	negative
Feterita	156 (26.9)	93 (16.0)	47 (26.3)	39 (21.8)
N13	116 (18.9)	205 (33.9)	47 (19.6)	99 (41.3)
Framida	74 (22.8)	147 (45.4)	11 (35.5)	14 (45.2)
2219B	181 (31.5)	127 (22.1)	137 (34.5)	109 (27.5)

Figures are totals of four replicate plates, figures in brackets expressed as a percentage of seeds germinated. Data for 'neutral' seedlings are omitted.

The above data were subjected to Chi-squared tests (using Yates'correction) and the results were found to be highly significant (probability $<.001$) when comparing resistant varieties of sorghum with susceptible varieties. The results comparing susceptible with susceptible and resistant with resistant were insignificant. Data from Zone 1 (0 - 1.5 cm from the stimulant source), which is probably greater than the maximum range of a Striga radicle, are highly significant. In Zone 2 the negative response to N13 stimulant was still significant whereas the response to Framida was no longer significant.

It appears therefore, that the susceptible varieties elicit at least some positive chemotropism, though not as much as might be expected, while the resistant varieties do not, and may conversely have a negative or repellant effect.

To appreciate the significance of this response it is useful to consider the geometric relationship between a Striga seedling and a host root in the soil. The chances of a Striga seedling germinating within a circle of known radius around a root of known radius and making contact with that root by chance can be calculated as in Table 3.

Table 3.
The chances of a Striga seedling making contact with a root, assuming random directional growth.

Radius of root (mm)	Striga seedlings within a radius of 1	2	3	4 mm
0.1	1/18	1/34	1/49	1/65
0.5	1/4	1/7	1/11	1/14
1	1/1	1/4	1/6	1/7

The figures in Table 3 assume no chemotropic response. Therefore, given the maximum range of a Striga radicle is 4-5 mm, and assuming there is stimulation of germination up to 4 mm, only 1 seedling out of 65 of those germinating within that radius will make contact by chance, with a root of 0.2 mm diameter and only 1 in 7 with a root of 1 mm diameter. Induction of a negative chemotropism by the host root exudate would further reduce the chances of the Striga making contact with a suitable host root, while the reverse would be true for any sorghum producing a positive response in the Striga. Relatively small differences in chemotropic stimulus could therefore have very large effects on "resistance".

Haustorial Initiation

The results presented in Tables 4 and 5 suggest that varietal differences are inconsistent and vary according to the section of host root used as a stimulant source. The area initiating the most haustorial development was the most mature section used, i.e. that taken from below the seed where it might be thought that the Striga would encounter some difficulty in penetrating the host. The actively extending section of the host root produced the next highest inducement of haustoria.

Table 4.
Induction of haustoria in seedlings of S. hermonthica (WRO 75-1 ex Sudan) by lengths of root from different root zones of four sorghum varieties.

No. with haustoria as % of those germinated (mean of 3 reps.)

Sorghum	Section of host root (cm from tip) Tip	1	2	3	4	below seed
N13	39.5	53.0	33.0	39.0	48.5	55.0
Feterita	51.0	DM*	50.0	30.0	50.0	80.0
2219B	62.5	45.5	27.0	40.0	36.0	79.0
SPV103	31.0	22.6	47.0	29.5	24.0	78.0
SEM = 17.8						

* D.M. data missing.

When root macerate was added to the Striga seeds simultaneously with the germination stimulant GR7, very high levels of haustorial initiation were observed, N13 stimulating at least as much as Feterita and 'clean' root initiating somewhat more than roots in contact with soil. When contact with root material was delayed for 36 hours after germination, haustorial initiation was reduced, particularly at the higher temperature of 33°C.

Table 5.

Induction of haustoria in seedlings of S. hermonthica (WRO 76-3 ex Sudan) by root macerates of two sorghum varieties.

Source of roots*	No with haustoria as % of those germinated		
	Root macerate and GR7 simultaneous	Root macerate added 36 hours after GR7	
Incubation temp.	33°C	33°C	23°C
N13 (soil)	79.0	19.7	34.5
N13 (clean)	98.8	20.4	36.3
Feterita (soil)	58.8	16.5	42.9
Feterita (clean)	83.9	24.4	38.6
Water control	0	0	0
SEM = 4.33			

* "soil" indicates roots from the side of pot mass, in contact with soil. "clean" indicates roots from the coil in the base of the pot.

On the basis of these experiments it would seem that low production of haustorial 'initiant' is not contributing to the resistance of sorghum variety N13.

Soil microflora

Sorghum variety N13 generally fails to show its resistance when grown in the "polybag" system using sterile procedures. Three experiments were conducted to determine if a more natural system, in which a soil microflora is present, would produce an expected differential between N13 and the susceptible variety Feterita. Results are presented in Table 6.

Table 6.

The development of S. hermonthica on the roots of two varieties of sorghum in 'polybags' as influenced by soil inoculum (3 expts.) and pseudomonads (1 expt. only)

Mean No. of Striga attached per bag (No. of replicates in brackets)

	Nutrient only		+ Soil inoculum		+ pseudomonads	
	N13	Feterita	N13	Feterita	N13	Feterita
Expt 1.	6.0 (4)	9.0 (4)	1.7 (3)	8.3 (4)	8.4 (3)	19.5 (4)
Expt 2.	2.0 (7)	8.0 (8)	4.9 (8)	17.1 (8)		
Expt 3.	19.8 (4)	6.8 (4)	5.6 (5)	26.8 (6)		
Mean	7.8	7.9	4.5	18.4		

The analysis of the data from the first experiment indicated that the addition of soil inoculum or pseudomonads had significant effects on the expression of resistance to Striga attack. While the differences between the cultivars in the nutrient (control) treatment were insignificant, both treatments caused a significantly ($P < 0.05$, using Student's T-test) greater number of Striga to develop on the susceptible variety Feterita. Numbers on N13 were not significantly increased by pseudomonads and were decreased by soil inoculum, hence the differential between the resistant and susceptible cultivars was significantly increased.

In the further two experiments, the increased success of Striga on Feterita in the presence of a soil flora was confirmed. On N13, soil again caused a decrease in Striga in experiment 3, but in experiment 2, numbers were very low in the nutrient control and in the presence of soil, numbers were increased to a level comparable with that in expts. 1 and 3. It is possible that sterility was not adequately maintained in the N13 nutrient control in Expt 2, with a consequent reduction in Striga attack comparable to or apparently even more effective than that caused by soil.

This series of experiments cannot be regarded as conclusive but it is strongly indicative that the resistance of N13 may be reinforced by certain soil microflora, while the susceptibility of Feterita is increased. Theoretically this could arise from a different balance of organisms being favoured in the rhizosphere of the two varieties, and those favoured by N13 including some with pathogenic potential on Striga. However, there could be other explanations relating to the factors discussed elsewhere in this paper. For instance, the microflora may influence the production of chemotropic stimulus, or the length, or susceptibility of the roots to attachment and penetration. Further studies are in progress to obtain a fuller explanation.

Root distribution

The results of the first set of observations showed little or no difference between N13 and Feterita in the growth rate of the primary roots. However, N13 produced only half as many lateral roots as Feterita and the total length of these roots averaged 63 mm per plant compared with 261.5 mm per plant for Feterita. So in the very early stages of development the susceptible Feterita was producing considerably more root length than was the resistant N13.

Table 7.
Plan area of sorghum roots at different "depths" in polyethylene bags.

Root area in transect 1.65 cm deep x 10.5 cm wide (mm^2)
(mean of 8 replicates)

Sorghum	Top 1	2	3	4	5	6	7	8	9	Bottom 10
N13	159	191	217	223	243	255	276	333	458	751
Feterita	194	277	315	352	447	541	567	621	798	1278
SEM = 41.90										

This was confirmed when plants from an experiment set up to examine the possible interactions of soil microflora and Striga infection were removed from the bags and the plan area of roots measured using an image analyser (Table 7). Differences were small between experimental treatments but there were distinct differences between the cultivars N13 and Feterita.

CONCLUSIONS

Previously, the search for resistant varieties has been mainly concentrated on one or two aspects of the life cycle of Striga, for example low stimulant production by the host plant or mechanical barriers within the host root offering resistance to penetration of the vascular system. It now appears that a number of other factors, acting separately, or collectively, can contribute to 'resistance' to Striga attack.

In particular there is shown to be an important potential for resistance by negative chemotropism (a repellant effect) or even by a lack of positive chemotropism as the chances of Striga seedlings making contact with the root by random can be quite small. The results of "in vitro" tests using root exudates separated into solvent rather than living roots, cannot be regarded as fully conclusive and further work is in progress to confirm the differences between varieties, using other more direct techniques, and to elucidate whether differences between varieties result from differential production of chemotropic substance, a masking effect of other inhibitory substances, or perhaps from different gradients of nutrient or pH in the rhizosphere.

Lack of ability to stimulate haustorial development of Striga does not so far appear to be a contributory factor in resistance of sorghum.

The sorghum variety N13 does not normally show its resistance when grown under sterile conditions in polyethylene bags and the results with deliberate innoculation with soil microflora suggest the resistance may be associated with and dependent upon a certain rhizosphere microflora. The same inoculum does not induce resistance in the susceptible varieties tested. This may indicate that the roots of different varieties favour the development of distinctly different floras, and that the flora favoured by N13 is either more directly pathogenic to Striga or otherwise interferes with Striga attack, perhaps even via an influence on chemotropism. The flora could, however, be the same in each case and affect the root systems differently. Further work is needed to clarify these possibilities.

Finally there is the possibility that quite simple differences in root morphology can contribute significantly to Striga attack. N13 is shown to have significantly less branching and hence length of root in the upper 10 cm depth of polybags, and comparable differences have been observed in pot-grown plants.

The possibility of resistance by mechanical barriers is not ruled out by these studies but we believe we have identified some further important characteristics which can contribute to resistance, which, if confirmed

could be utilised in a resistance breeding programme. All the techniques used give results in a relatively short period of time (the longest time span being about four weeks for the polyethylene bag experiments) and involve the use of simple and readily available materials. The use of such techniques in screening programmes could speed up the identification of 'resistant' traits in a wide range of cultivars.

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TECHNIQUES FOR SETTING UP POT EXPERIMENTS INVOLVING THE PARASITIC WEED STRIGA

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Abstract The basic techniques used at the Weed Research Organization (WRO) to set up and execute pot experiments involving the parasitic weed *Striga* are described. Variations in technique are presented and discussed with regard to obtaining uniform growth of the host, predictable and uniform levels of *Striga* infection and differential behaviour of resistant and susceptible host varieties. Factors that are of particular importance include temperature, light intensity, numbers of *Striga* seed per pot and interactions of soil fertility (especially nitrogen) with pot size.

INTRODUCTION

The Weed Research Organization (WRO) at Oxford U.K. has had an interest in *Striga* for over twenty years. The earlier pot experiments were mainly concerned with controlling the parasite by herbicides (Parker, 1974), but in more recent years experiments have been related to varietal resistance of the host plant (Parker and Reid, 1980).

Over the years much experience has been gained in obtaining some control over the growth of a relatively difficult experimental plant. The aims and objectives of setting up pot experiments with *Striga* are to obtain a) uniform growth of the host, b) predictable and uniform levels of *Striga* infection, c) differential behaviour of susceptible and resistant varieties.

There are many factors which influence these aims and objectives and play a part in the success of an experiment. The basic techniques used at WRO for setting up and assessing an experiment are presented, and the factors involved in producing the right conditions to fulfil the aims and objectives are discussed.

METHODS AND MATERIALS

The basic procedures

These have changed little over the last twenty years. The soil comes from two local sources, Begbroke Hill and Yarnton. Both samples are very similar sandy loams with a pH of 7.5. The soil is shredded and riddled to remove any large stones and vegetation, and then nutrients are added to the soil at the following rates: P as 18% super phosphate at 2.0 g/kg; K as 48% potassium sulphate at 1.2 g/kg ; Mg as commercial magnesium sulphate at 0.8 g/kg; N as ammonium sulphate at 0.1 g/kg, plus trace elements.

Clean black plastic, free-draining pots of 12 or 15 cm diameter are partly filled with standard soil, such that the top 7 cm of the pot is left empty. This is then topped up with a mixture of soil + *Striga* seed.

Samples of *Striga* seed are collected from the field and cleaned well before use. This is achieved by passing the seed through a series of fine mesh sieves with apertures of 250 and 125 µm. The seed is then given a WRO code number (e.g. 75-1, indicating the first collection of that species in 1975), and stored in labelled glass containers under ambient laboratory conditions until required for use. Periodically all samples of seed are subjected to tests to ascertain viability, and percentage purity. Seeds are measured in a series of 10 graded scoops, made by drilling holes of different depth and diameter in small metal bars, which progress from 4-44 µl volume, holding approximately 550 - 6,400 seeds. Knowing the germination percentage and the amount of rubbish present in the samples, use of the scoops allows control of seed numbers from pot to pot or treatment to treatment and between different experiments.

The *Striga* seed is mixed into the soil in one of three ways. (i) For large quantities of the same *Striga* seed, the measured amounts of soil and *Striga* seed are blended in a cement mixer for three minutes. For smaller quantities of soil, the measured amounts are mixed by either (ii) shaking soil and seed with trapped air in a large polyethylene bag for two minutes or, (iii) by passing the soil and seed four times through a large funnel. All these methods give a good even mix of seed in the soil.

The filled pots are placed on the glasshouse bench in the required statistical design at a density of 30 - 36 pots/m^2. Seeds of the host plants are sown three to a pot at a depth of 1.5 cm. During all operations great care is taken to keep the equipment clean in order to prevent any carry-over of different *Striga* seed from one treatment to another. The glasshouse temperature is set at 24/18°C day/night regime, and the pots are lightly watered as required.

The host plants emerge in three to four days. They are thinned to one plant/pot, and any transplanting necessary to fill the gaps completed within the same treatments. After three weeks the temperature regime in the glasshouse is altered to 30/22°C. i.e. a minimum of 30°C in the daytime, with the temperature allowed to rise over 30°C periodically by solar heating. This encourages germination of the *Striga* seed following the period of 'pre-conditioning'. The first *Striga* will begin appearing above ground from five to seven weeks after setting up the experiment. The experiments are maintained for 12-15 weeks, allowing two sets of experiments during the growing season from March to November.

Assessment of experiments

During the course of an experiment the growth and development of the host and parasite are monitored regularly. The most important of these assessments is a weekly count of the *Striga* plants as they emerge from the soil. This, together with observations on the growth of the host such as stunting, blotching of the leaves and general vigour, gives an early

indication of the effect of *Striga* on the host.

Other assessments are made according to the type of the experiment. A destructive harvest of one or two replicates at intervals throughout the growing period allows for a more detailed quantitative assessment of the effect of *Striga* on the shoot and roots of the host, and reveals any differences between the treatments as they begin to develop. These assessments usually take the form of measuring, weighing or counting different parameters of plant growth, often in conjunction with more detailed laboratory assessments.

The following parameters are measured when making the final assessments at the conclusion of the experiment.

Assessment of shoot growth. The height of the host gives an indication of the reduction in vigour caused by *Striga*. The height of the growing point gives a more accurate measurement of the effect of *Striga* on the host, in terms of its potential to produce a flowering head, and therefore its potential yield. The fresh weight of the shoot is not always considered accurate, especially if the pots have not been watered evenly, but it gives an indication of differences in growth. The dry weight of the shoot is more accurate but also more time consuming, and may not always be necessary. These three (or four) parameters are essential in assessing shoot growth, but other parameters such as the number of tillers, and the number and length of green leaves etc. may be made in more detailed assessments.

Assessment of host roots can be time consuming because very careful washing is necessary for detailed measurements. At WRO the standard practice is to turn out all the pots after the shoots have been harvested, and score the root vigour on a scale of 1-7. The pattern of root development may also be noted. These observations give a subjective indication of the general root growth, and more detailed observations including root dry weights are made on any treatments or varieties which these observations or scoring indicate may be of special interest.

Assessment of Striga. The numbers of emerged *Striga* plants per pot are assessed throughout the growing period and the final count is taken as the pots are harvested. Other parameters which are measured include the height of the three tallest *Striga* and the number flowering. This shows the vigour of the *Striga* alongside the numbers per pot, which together indicate the scale of the attack. Fresh weight of *Striga* is the more practical parameter to measure, as in some cases the *Striga* are too few or too small for easy dry weight assessment. Counting attached *Striga* below ground is possible in the early stages, with careful washing, but by the time of 'harvest' at 12-15, weeks the density of root and force of washing required make it impracticable.

All data recorded are subjected to analysis of variance. *Striga* counts are usually first transformed to log data. Variations of these standard methods are noted in the following section.

RESULTS OF VARIATIONS IN TECHNIQUE

Soil type

Striga is traditionally thought to be more prevalent on dry sandy soils but experiments at WRO have shown that there were no advantages to be gained in using a sand:soil mix over the normal sandy loam as far as it affected the emergence of *Striga*, as shown in Table 1. As plain soil is a more convenient substrate this continues to be the standard at WRO.

Table 1
Effect of two soil types on the number of emerged *Striga* on sorghum var. YE 90L

Soil Type	No. of *Striga* emerged (Mean/pot)*		
	Experiment 1		Experiment 2
	S. asiatica (WRO 75-1)	*S. hermonthica* (WRO 77-25)	*S. hermonthica* (WRO 79-4)
Plain soil	1.59	1.46	0.96
Soil : Sand 3:1	-	-	0.93
Soil : Sand 2:1	1.21	1.18	-
Soil : Sand 1:1	-	-	1.09
LSD (P=0.05)	0.21	0.21	0.35

* Data transformed to log

Placement of seed in the soil and numbers of seed per pot

Table 2 shows the results of an experiment involving seven different ways of placing two different species of *Striga* seed in the pot. It appeared that placement of seeds in the soil was not critical, but that shallower placement resulted in larger numbers of plants emerging, even at the lowest density of seeds sown. As the numbers emerging were sufficient in the treatment most convenient to set up, i.e. mixed in the top 6 cm, this method has been adopted for use at WRO.

Table 2
Striga emergence at different placements and different densities in the soil on Sorghum var. YE 90L.

Placement of seed in soil	No. of *Striga* emerged (Mean/pot)*					
	S. hermonthica (WRO 77-25)			*S. asiatica* (WRO 75-1)		
	400	1600	4800	500	2000	6000 +
Mixed in top 6 cm	1.22	1.35	1.23	1.27	1.71	1.06
Mixed in top 3 cm	1.42	1.55	-	1.24	1.49	-
Mixed in 3-6 cm layer	1.44	1.28	-	0.62	1.37	-
Layer at 1 cm depth	1.27	1.56	-	1.12	1.11	-
Layer at 3 cm depth	1.19	1.33	-	1.07	0.84	-
Layer at 5 cm depth	1.01	0.68	-	0.69	0.34	-
Ring round edge at 3 cm	0.85	1.25	-	0.96	0.99	-
LSD (P=0.05)			0.31			

* Data transformed to log. + = Nos. of seeds sown per pot.

Sufficient numbers of *Striga* emerged from the lowest numbers of seed per pot, and there was no proportional increase in emergence with the higher seed numbers and no difference between the two different species. A second experiment to look at the density of seeds per pot compared two samples of *S. hermonthica* of different ages from the same site at Abu Naama, Sudan. Although the plants emerged more slowly in the newer seed sample, the final number emerged, and their fresh weight, correlated directly with the initial amounts of seed sown. The mean number of emerged *Striga* per pot, together with fresh weight per pot are presented in Table 3, as transformed data. The emergence of the older seed sample followed a pattern similar to the same seed sample in the earlier experiment, (see Table 2), and a plateau of emergence was reached at 400 seeds per pot.

Table 3.

Emergence and shoot fresh weights of two *Striga hermonthica* samples sown with sorghum var. 2219B at five different densities.

Nos seeds/pot	*S. hermonthica* (WRO 77-25)			*S. hermonthica* (WRO 79-8)		
	No/pot	(log)	Fresh wt.(g)	No/pot	(log)	Fresh wt.(g)
200	10.2	(0.97)	2.58	1.2	(0.25)	1.24
400	19.5	(1.24)	2.87	3.0	(0.46)	1.40
800	15.8	(1.19)	2.99	8.8	(0.68)	1.74
1600	20.2	(1.30)	3.05	13.8	(1.12)	2.93
3200	23.2	(1.32)	3.12	29.8	(1.48)	2.75
LSD (P=0.05)	-	(0.36)	0.72	-	(0.36)	0.72

Soil fertility

Experience at WRO has shown that a level of N equivalent to 0.1 g/kg ammonium sulphate gives satisfactory growth of the host with good *Striga* emergence in 15 cm pots, but this level of N leads to weak host growth in smaller 12 cm pots. In the smaller pot size the differential between susceptible varieties and those with resistance based on low stimulant production, such as Framida, is generally well demonstrated. Varieties such as N13, however, which have field resistance based on as yet poorly defined mechanisms (see Dixon & Parker, 1984) often support abundant *Striga* emergence in the smaller pots at low N, and differential behaviour is often demonstrated better at higher N levels.

Table 4 shows the effects of different levels of N on the emergence of *S. hermonthica* on two sorghum varieties, through the growing period. The higher levels of N delayed the emergence of *Striga* somewhat on the susceptible Feterita but numbers were eventually highest at the highest N level. The delay in emergence due to high N was more prolonged on N13 resulting in much greater differential between the two varieties. Similar results were obtained with other samples of *S. hermonthica* from West Africa and *S. asiatica* from both India and South Africa. The lack of prolonged suppression of *Striga* on the susceptible variety can be understood when the very limited volume of soil (about 1ℓ) in a 12 cm pot and hence the limited

amount of nutrient available for the host, is considered.

Table 4.
Effects of three different levels of nitrogen on emergence of *Striga hermonthica* (WRO 75-1) on two sorghum varieties N13 and Feterita.

Weeks	7	8	9	10	11	12	13
Low N (0.1 g amm. sulphate per kg)							
Feterita	0.57	1.16	1.23	1.23	1.27	1.26	1.26
N13	0.18	0.47	0.74	0.89	1.01	1.10	1.17
(Ratio Fet:N13)*	(2.4)	(4.8)	(3.1)	(2.2)	(1.8)	(1.4)	(1.3)
Medium N (0.3 g amm. sulphate per kg)							
Feterita	0.00	0.56	0.88	1.05	1.18	1.22	1.24
N13	0.00	0.12	0.43	0.60	0.51	0.56	0.73
(Ratio)	-	(2.8)	(2.8)	(2.9)	(4.7)	(4.5)	(3.3)
High N (0.9 g amm. sulphate per kg)							
Feterita	0.00	0.57	1.05	1.24	1.37	1.46	1.49
N13	0.00	0.00	0.00	0.15	0.36	0.55	0.89
(Ratio)	-	∞	∞	(12.4)	(10.2)	(8.0)	(4.0)
LSD (P=0.05)	0.12	0.21	0.25	0.28	0.28	0.29	0.31

* based on actual numbers: other data are log transformations.

Analysis of the soil before and after an experiment using the 'standard' additions in 12 cm pots showed that total nitrogen was depleted by 70% The nitrate N was reduced by about 50% but the more available ammonium nitrogen was depleted by up to 95%. Phosphorus and magnesium were depleted by about 50% and potassium by about 75%. The rate of nutrient depletion can be reduced by using the larger 15 cm diameter pots (2ℓ capacity). It is then generally best to keep to the low N (0.1 g ammonium sulphate per kg) level. With the larger pots and higher N the vigour of the host shoot tends to create excessive shade and hence a further suppression of *Striga* unless pots can be spaced out more widely which requires a greater area of potentially expensive glasshouse space.

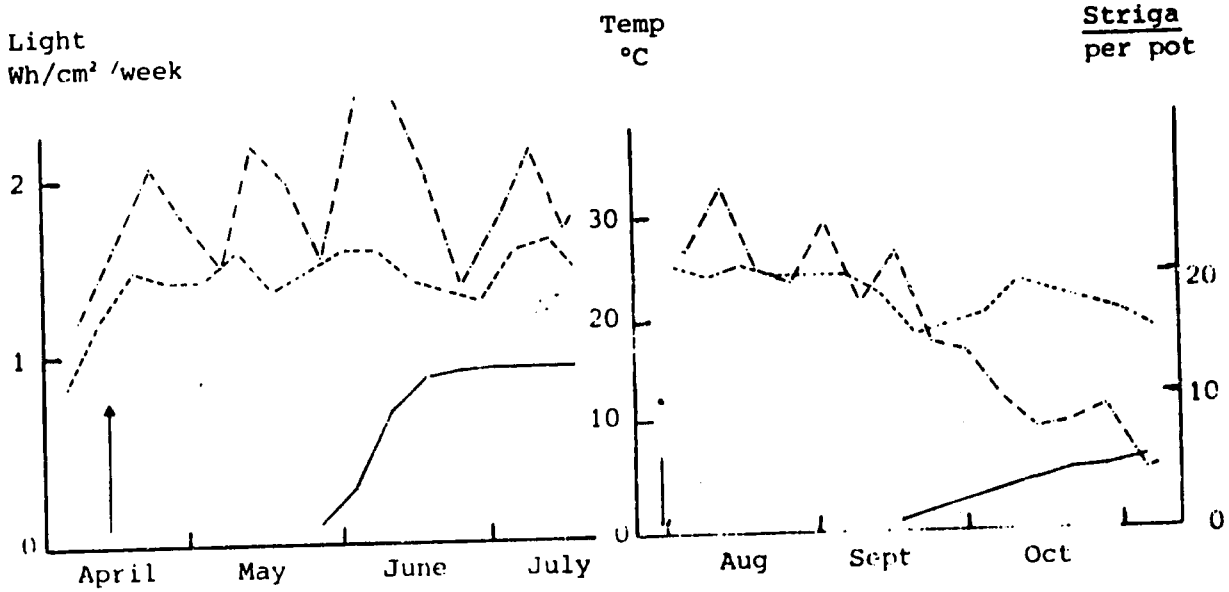
Temperature and light and the effect of season

Figure 1 illustrates the characteristic trends in light and temperature through the season in which two experiments are normally conducted at WRO. It may be seen that there is a tendency for *Striga* emergence to be lower in the second half of the season. The results illustrated are from different experiments but are comparable in terms of soil, pot size, *Striga* samples and sorghum variety, and are generally typical, though differences are not always so great.

A difference not clearly shown in the figures is a tendency for the temperature in the early part of the season in April, to be relatively low and constant. In August the mean is comparable, but there is greater fluctuation, which includes temperatures over 30°C. This might be expected to favour *S. asiatica*, which can benefit from higher pre-conditioning

temperatures (Reid & Parker 1979). This however is not the case and suggests the lower emergence of *S. hermonthica* in the latter half of the season is due either to the slightly lower temperatures from 5 to 6 weeks after sowing, or, more probably to the steeply decreasing light availability after this time. It is normally supplemented from mid-September, with high pressure sodium lighting but the intensity of this light at about 0.34 Wh/cm^2/week which is inadequate to compensate for the decrease in natural light.

Figure 1. The effect of season on the emergence of *S. hermonthica* on sorghum var. 2219B in 1982.



— — — Light available in glasshouse (Watt-hours/cm^2/week) based on outside data and assumes a 60% loss through glass etc.

— — — — Weekly mean temp (°C)

——— *S. hermonthica* (WRO 75-1, 77-38, 77--39). ↑ date of sowing

DISCUSSION

The above selected results illustrate a few of the more important factors to be considered or controlled when working with *Striga* in a temperate environment, where field work is not possible. Some of the conclusions, however, may be relevant also to the conduct of pot experiments within the tropics.

The results with soil type have been very limited but are in keeping with the conclusions of Nelson (1958) and Robinson (1960) working with *S. asiatica* on a wider range of soils in USA, and tend to confirm that soil type should not be a critical factor.

Similarly the exact placement and distribution of seed in the soil is not critical. Emergence tends to be greater from shallow-sown *Striga* seed,

but mixing to a depth of 6-7 cm appears to provide a reasonable compromise between maximising emergence and representing natural depth distribution in the field.

The number of seeds per pot requires careful adjustment. In both experiments on seed numbers, it was shown that with certain seed samples, increasing numbers of seeds sown beyond 400 per pot led to no proportional increase in emergence. This was probably due to excessive intra-specific competition and failure of seedlings to reach the soil surface. Conversely it may be deduced that if excessive seed is used, treatments causing substantial reduction in germination or attachment of *Striga* might show no less emergence than untreated. Such an effect could lead to reduced differential between susceptible and resistant varieties.

The optimum number of seeds per pot may often be greater than 400 where viability and virulence are lower. The different behaviour of the younger of two seed samples from the same site (Table 3) can be explained on the basis that it was slower to pre-condition and had a lower final viability. The later development allowed stronger growth of the host which in turn supported a greater eventual emergence of *Striga*.

Striga species thrive in soil of low fertility, and the more specific effect of nitrogen in reducing the incidence of *Striga* attack is well documented, (e.g. Last, 1960; Ogborn, 1970; Bebawi, 1981). This effect also has been confirmed in pot experiments by Younis and Agabawi (1965) and Yaduraju *et al* (1979). It is not clear exactly how increased nitrogen delays and decreases the emergence of *Striga*. Various possibilities are discussed by Parker (1984a,b) and Dixon and Parker, (1984). Whatever the reasons it is clear that nitrogen must be kept at a sufficiently low level in pot experiments to result in a suitable host/parasite relationship.

Unfortunately, with limited glasshouse facilities it is not practicable to use large soil containers and in the typical size of pot used (12 to 15 cm diameter) the soil volume is small and depletion of nutrients by the potentially large host, sorghum, is rapid in the course of the experiment. The initial low level of N (0.1 g ammonium sulphate per kg soil) is in fact quite high in terms of a field concentration, but is rapidly depleted and becomes limiting before the experiment is completed. For many types of experiment the standard low level and 12 cm pots are adequate, but for varietal resistance studies a greater availability of N is preferable, either by using a higher concentration in small pots, or the low concentration in larger (15 cm) pots. The greater differential between susceptible sorghum varieties and those with resistance of the N13 type has not always been consistent under the higher N conditions and there is still much to be learned of this interaction. Experience has shown that nitrogen applied in split applications was detrimental to the emerged *Striga*, so this is not a practical answer to keeping the host supplied, and it is preferable to incorporate all nutrients at the outset of the experiment.

There is little indication from the literature that other nutrients have significant influence on the incidence of *Striga* attack. Saunders

(1933) reported no benefit in the control of *S. hermonthica* from applications of superphosphate, and Last (1960) found that neither phosphorus nor potassium affected the incidence of *S. hermonthica* attack or improved the yield of sorghum. Relatively little has been done at WRO to vary these other nutrients, but no consistent differences have been observed which suggest that they are critical in any way.

Striga is generally expected to thrive in low or erratic rainfall areas (Ogborn, 1970). Deliberately restricting the watering of pot experiments has not generally led to increased *Striga* development, and in a single experiment in 1980 it was shown that even continuous saturation of the soil did not reduce the emergence of a very virulent strain of *S. hermonthica* (WRO 75-1) from Shambat in Sudan. Hence watering method or intensity is not thought to be a critical factor, for the purposes of normal experiments.

Working with *Striga* in a temperate zone during the summer when day length may be up to 18 hours, raises questions concerning possible photoperiodic effects. However, Kust (1964) looked at the effect of photoperiod on the growth of *Striga*, in pot trials using growth chambers, and showed that sorghum was successfully parasitised by *S. asiatica* under a range of photoperiods up to 17 hours. He concluded that photoperiod was not critical in the germination and development of *S. asiatica*. The total availability of light seems to be more important than the photoperiod alone, and the host plants do not grow well, even with supplementary lighting, during the months of November to March. The cost of maintaining adequate temperature during that period would in any case be prohibitive.

Over the period April to October, it is possible to complete two series of experiments, each lasting 12-15 weeks. The second half of the season provides less ideal conditions, perhaps because of some differences in temperature pattern, which could be controlled more carefully, but more probably because of declining light availability (intensity and day length) after the middle of September, which cannot be fully compensated by supplementary artificial lighting. The importance of a strong flow of photosynthates to the root system, for good *Striga* development is emphasised elsewhere by Parker (1984b), and it is highly probable that after mid-September, the flow is simply not adequate to sustain rapid growth and emergence of *Striga*. Useful experiments are still possible at that time of year, but interpretation of results has to be a little more cautious. For instance, a treatment that delays *Striga* germination or development for just a few weeks may appear to cause a more complete suppression at that season than in an April to July experiment.

Experience gained at WRO in the basic experimental techniques is generally confirmed in the literature by reports from the field and glasshouse. These techniques form a basis for the experimenter to use when setting up pot experiments designed to understand more fully the complicated relationship of *Striga* with its host.

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INHERITANCE OF STRIGA GESNERIOIDES RESISTANCE IN COWPEA

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Abstract. Damage caused by Striga gesnerioides (Willd.) Vatke. in cowpea (Vigna unguiculata L. Walp.) is becoming serious in the semi-arid regions of Africa. Resistant varieties are the best solution to overcome this problem. Cowpea germplasm was screened to identify such varieties, and two varieties, SUVITA-2 and 58-57 have been found to be resistant. SUVITA-2 was crossed with a local (Kaya local) as well as an improved (KN-1) variety to find out the mode of inheritance of this character. A single dominant gene was found to be controlling this resistance in the cross between SUVITA-2 x Kaya local, whereas the data from the cross between SUVITA-2 x KN-1 fitted a 9:7 ratio of susceptible and resistant plants. More studies are underway to further elucidate this problem.

INTRODUCTION

Striga gesnerioides (Willd.) Vatke. is a parasitic plant that causes severe damage to cowpea (Vigna unguiculata L.Walp.) crop in the Semi-Arid zones of Africa. Obilana (1983) observed it to be spreading rapidly southward in Nigeria, from its traditional area of occurence in the Sahel zones and Sudan savanna to Northern Guinea savanna. Striga damage is expected to further increase with increased acreage of cowpea cultivation in Nigeria and other neighboring countries as a result of high population pressure, which disrupts the traditional crop sequences, reduces the duration of natural fallow and obliges farmers to use the same piece of land under the same crop year after year. It is, therefore, imperative to develop Striga resistant cowpea cultivars as this may provide a cheap means of protecting this crop against severe yield losses. To achieve this, information on resistant source (s) and mechanism of inheritance is basic. This paper deals with such information on cowpea Striga.

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MATERIALS AND METHODS

1. Confirmation of Striga resistance in cowpea

Experiments were conducted both in the field and in the screenhouse to confirm resistance to gesnerioides found earlier in the cultivars SUVITA-2 and 58-57 (SAFGRAD/IITA Annual Report, 1981). For the field experiment, a chess board design (Vasudeva Rao et al, 1981) was used where both SUVITA-2 and 58-57 were planted in such a way that they were always surrounded by a susceptible check cultivar, KN-1. In the screenhouse, individual plants were planted in pots, and sixteen pots were used for each of the three cultivars, SUVITA-2, 58-57 and KN-1. Eight of these pots were artificially infested with Striga seeds whereas the other eight were not infested and were used a controls. Striga emergence was recorded on individual cowpea plants, and then per cent infested plants with Striga were computed for each cultivar.

2. Evaluation of promising selections for resistance to Striga

Prior to 1981, a cross involving SUVITA-2 (resistant to Striga) and TVu 2027 (susceptible to Striga but resistant to bruchids) was made to develop bruchid resistant materials. Selections from this cross were made for seed quality, and the 21 most promising were bulked and evaluated for Striga resistance and were further evaluated in 1983 alongwith susceptible (Mougne, TN 88-63 and Kaya Local) and two resistant (58-57 and SUVITA-2) cultivars. The experimental design was a randomized complete block with three replications and the plot size was 4 rows, 4 meters long. The trial was planted in a Striga sick plot which was artificially reinfested to ensure a uniform distribution of Striga infestation.

3. Study of inheritance of Striga resistance

To get information on resistance to Striga in cowpeas, SUVITA-2 was crossed with Kaya Local and KN-1. Kaya local is reported to be highly susceptible. Striga emerges fast where it is grown and can prevent its flowering. KN-1 has some level of resistance to Striga ; it delays Striga emergence (SAFGRAD/IITA Annual Report 1982). Parents, F1, F2, BC1 and BC2 generations of these crosses were evaluated in a Striga sick plot, which was artificially reinfested. The materials originating from KN-1 x SUVITA-2 cross were evaluated in the 1982 crop season, and those from Kaya local x SUVITA-2 in 1983. The observations were taken on a single plant basis and any cowpea plant showing presence of Striga was considered as susceptible.

RESULTS AND DISCUSSION

Table 1 shows the response of cowpea cultivars to artificial infestation of Striga both in the field and the screenhouse experiments. Only 13% plants of SUVITA-2 (in 1982) and 38% of 58-57 (in 1983) were found infested with Striga in the screenhouse experiments. This contrasted to over 60% plants of KN-1 both in the field and in the screenhouse in 1982, and 100% plants of Kaya Local in the screenhouse in 1983. These results suggested that SUVITA-2 and 58-57 have a high level of resistance to Striga, whereas Kaya Local is highly susceptible and KN-1 has a moderate level of resistance.

Table 1. Response of cowpea cultivars to Striga infestation under field and screenhouse conditions at Kamboinsé, Upper Volta.

Variety	Per cent plants infested with Striga				
	Field (sick plot)	Screenhouse (pots)			
		Infested		Control	
	1982	1982	1983	1982	1983
SUVITA-2	0	12.5	0	0	0
58-57	0	0	37.5	0	0
KN-1	66	63	-	0	-
KAYA LOCAL	-	-	100	-	0

The response of promising selections from the SUVITA-2 x TVu 2027 cross is shown in Table 2. Striga emerged minimum 5 days later in the plots of these selections as compared to susceptible cultivars. Per cent Striga infestation was lower and grain yield higher among them as compared to susceptible cultivars, except TN 88-63. They were similar to resistant varieties in time of Striga emergence, per cent infestation by Striga and grain yield. These results suggested that they were resistant to Striga.

Table 2. Responses of cowpea selections and cultivars to artificial Striga infestations at Kamboinsé, Upper Volta, 1983.

Cultivars	Days to 50% flowering	Days to first Striga emerg.	% with Striga emerg.	Grain yield kg/ha
Promising selection				
KVx 30-141-1G	45	42	1.0	1469
KVx 30-166-3G	44	£	0.0	1408
KVx 30-183-3G	44	£	0.0	1567
KVx 30-199-4G	46	37	11.0	1397
KVx 30-312-3G	44	35	7.7	1289
KVx 30-323-1G	46	36	10.3	1266
KVx 30-403-1G	44	36	2.7	1247
Susceptible check cultivars				
Mougne	40	30	87.3	826
TN 88-63	41	30	82.3	1322
Kaya Local	63	29	63.3	34
Resistant cultivars				
58-57	42	42	1.0	1229
SUVITA-2	43	£	0.0	1486
Mean	45	26	22.2	1212
C.V. %	2.3	n.a	47.7	19.1
LSD 5%	1.6		17.4	370

£ = Striga did not emerge.

Table 3. Segregation for Striga resistance in different populations of crosses of KAYA LOCAL x SUVITA-2 and KN-1 x SUVITA-2 at Kamboinsé, Upper Volta.

Lines or crosses	Total		Segregation Ratio		X^2 Value	Probability of a greater value
	Total	Infested	Susceptible	Resistant		
Cross : Kaya Local x SUVITA-2						
Kaya Local	75	66	1	0	1.08	0.50-0.25
SUVITA-2	78	0	0	1	0.00	1.00-0.90
F1	77	7	0	1	0.63	0.50-0.25
F2	242	71	1	3	2.19	0.25-0.10
BC1 (Kaya Local x F1)	107	48	1	1	1.34	0.25-0.10
BC2 (SUVITA-2 x F2)	373	20	0	1	1.19	0.50-0.25
Cross : KN-1 x SUVITA-2						
KN-1	82	80	1	0	0.49	0.90-0.75
SUVITA-2	80	5	0	1	0.31	0.75-0.50
F1	57	52	1	0	0.44	0.75-0.50
F2	211	128	9	7	3.23	0.10-0.05
BC1 (KN-1 x F1)	140	136	1	0	0.11	0.75-0.50
BC2 (SUVITA-2 x F1)	260	93	1	3	16.0	0.005-0.00

The response of cultivar TN 88-63, which reacted like a susceptible plant (early and high per cent of Striga emergence) but produced a very good yield, could be an expression of tolerance which probably could be justified from its origin at Maradi, Niger, a heavily Striga infested area.

Table 3 shows the segregation for Striga resistance in different populations of Kaya Local x SUVITA-2 and KN-1 x SUVITA-2 crosses. The cross between Kaya Local x SUVITA-2 showed a 3:1 ratio, suggesting that the resistance was simply inherited and controled by a single dominant gene. Whereas, the cross between KN-1 x SUVITA fitted a 9:7 ratio. This could be attributed to non-allelic interaction. It should be pointed out that KN-1 has shown some level of resistance to Striga by delaying its emergence as compared to Kaya Local (SAFGRAD/IITA Annual Report, 1982). And parents with similar response when crossed can produce a different F1 progeny (Bateson, 1913). This was probably what happened in the case of KN-1 s SUVITA-2 cross, where the F1 generation was susceptible to Striga. More work is already underway to further elucidate the type of gene action and the mode of inheritance of Striga resistance in cowpeas, however, it was quite clear that the resistance was genetically controlled and could be transfered. For this reason, SUVITA-2, is continued to be used to transfer Striga resistance into other improved cultivars.

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A REVIEW OF CULTURAL CONTROL OF STRIGA HERMONTHICA IN SUDAN

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Abstract. Cultural control of Striga in Sudan is reviewed. Current practices include crop rotation, crop spacing, sowing dates, nitrogen application, weeding, planting of resistant varieties, and catch and trap cropping.

INTRODUCTION

Sorghum [Sorghum bicolor (L.) Moench] is the predominant cereal of Sudan where it provides more food and drink for man and more sustenance for beast than all crops together. Throughout Sudan there are varieties to suit all climates and conditions (Bacon, 1948). Being mainly a peasant's crop for home consumption, sorghum does not figure as largely in international trade as cotton (Gossypium spp.), millet [Pennisetum typhoideum (Burm.) Stapf. and Hubbard], groundnuts (Arachis hypogea L.), or sesame (Sesame orientale L.)

Sorghum as well as millet, rice (Oryza sativa L.), and sugarcane (Saccharum officinarum L.) is a host crop of the parasitic weed Striga hermonthica (Del.) Benth. Where a frequently recurring sorghum or millet crop is required, Striga multiplies quickly with serious effects. Several workers reported losses of grain sorghum yields up to 70% as a result of Striga infestations (Basinki, 1955; Doggett, 1970; Last, 1961; Bebawi and Farah, 1981b). However, under severe infestations there may be no yield and the host plants killed (Andrews, 1945).

Chemical and cultural practices are the main measures of control of Striga in the Sudan (Andrews, 1945, 1947; Wilson - Jones, 1952, 1953a, 1955; Basinki, 1955; Tarr, 1957, 1961; Agabawi and Younis, 1965; Younis and Agabawi, 1965; Bebawi, 1981b; Bebawi and Farach, 1981a,c; Babiker and Hamdoun, 1982, 1983; Babawi and Abdel Aziz, 1983; Bebawi and Elhag, 1983). However, agriculturists in Sudan have more experience with cultural than chemical control of Striga. Cultural control measures of Striga in the Sudan are to a great extent influenced by the edaphoclimatic factors. Ungerminated Striga seed are reported to remain viable in the soil for at least 5 years (Andrews, 1948).

The soils of Sudan are predominantly sandy with little water holding capacity in the North; heavy almost impermeable alkaline clays in the center and parts of South Sudan; and more permeable acid red ironstone soil in the South (Andrews, 1948). At any one time 40% of the total cropped area of Sudan is under sorghum with 37% under rainfed agriculture. Sorghum is reported to have a high requirement for nitrogen, therefore little available nitrogen is usually left in the soil after a sorghum crop (Rai, 1961).

A. CULTURAL CONTROL OF STRIGA IN THE RAINLANDS

1. East Sudan

Observations on the heavy clays of East Sudan on rainland have shown that as the land becomes progressively infertile owing to continuous cropping with sorghum, the infestation with Striga becomes increasingly severe until a stage is reached when it becomes no longer profitable to grow sorghum. In East Sudan, new land claimed from both forest or bushland and grassland for cultivation is described in Arabic as 'Kurd' land. This same land in its second, third, and fourth year of cultivation is referred to in Arabic as 'Kamadob', 'Dahr Kamadob' and 'Gassaba'. The type of cultivation in lands ranging from 'Kurd' to 'Gassaba' is associated with the intensity of Striga infestations and soil fertility. The 'Kurd' land is characterized by the least intense infestations of Striga and greatest soil fertility whereas the 'Gassaba' is the most infested and least fertile land. Farmers of East Sudan cultivate the 'Kurd' land with the sorghum variety 'Feterita', the 'Kamadob' with the variety 'Red mugud', the 'Dahr Kamadob' with the variety 'Safra' and the 'Gassaba' with sesame. Thus, in East Sudan a 4-yr rotation system has developed as a cultural measure of control against Striga. This system selects the most susceptible variety 'Feterita' to lead the cycle on the assumption that the high soil fertility would improve the general physiological performance of the host crop and thereby induce some form of physiological resistance to the early maturing 'Feterita' crop (2 1/2 to 3 1/2 months). However, irrespective of host crop variety, grain production declines with years of cultivation. In this cycle, sorghum cultivation is discontinued after the third year because the intensity of Striga infestation would have reached a critical level in the soil so that further cultivation with sorghum becomes uneconomical. Sesame is the fourth year crop because of its low requirement of nitrogen, immunity to Striga and cash value.

In East Sudan late sowing (31 August onwards) of sorghum is preferred to early sowing (June 9 to August 17) because the intensity of Striga infestation is much less with late than with early sown sorghum. The late sowings occur when the rains are well advanced and the soil temperatures have become relatively cool whereas early sowings occur when the rains are intermittent and the soil temperatures are relatively warm. Thus the drying and wetting of warmer soils of early sowings may be conducive to the greater intensities of infestations of Striga whereas the continuously wet cool soils of late sowings may be conducive to less intense Striga infestations.

2. West Sudan

In West Sudan a different cultural control of Striga to that of East Sudan is practiced. In the clay lands of West Sudan generally a change of crop on an infested field from sorghum to Pennisetum millet would result in a Striga free crop of the latter where the farmer could no longer grow sorghum successfully. It has also been observed that on the Kordofan sands where Pennisetum millet is very heavily attacked, sorghum will give

a crop on fields too heavily infested with Striga for the successful cultivation of millet. In West Sudan the situation on the sandy areas is the reverse of that in West Africa, for it is a change from Pennisetum to sorghum that reduces the Striga infestation (Wilson - Jones, 1955).

A West African experience with Striga was reported by Porteres (1952) as "the debasement of the edaphoclimatic complex" attempts to explain the cultural control of Striga in West Africa. According to this theory, native agriculture follows a course of deterioration from the first opening of new land. Starting with the cultivation of sorghums which are presumed to have the highest fertility requirements, a stage is reached when soil exhaustion results in a seriously weakened crop which then becomes susceptible to Striga attack. A change to a crop with lower fertility requirements, i.e., Pennisetum millet, then results in an almost Striga free crop, although the preceding sorghum might have been heavily attacked. After some years, during which further exhaustion of the land occurs, The Pennisetum millet yield becomes less and less until eventually Striga again appears. Finally, the Pennisetum millet is replaced by Digitaria exilis which has a lower fertility requirement (Porteres, 1952). When the soil is too exhausted for this crop as signified by a heavy Striga attack, no further cultivation is possible and the land must be abandoned.

However, in West Sudan, Wilson - Jones (1955) has shown that Striga does not only appear as a result of poor soil conditions because on the clay soil of Sudan the use of herbicides to control Striga has considerably increased crop yields. Nonetheless, he admitted that soil exhaustion is a serious factor which is bound to occur under continuous cropping without manure especially on the sandy soils (Wilson - Jones, L., 1955). Wilson - Jones argued that it is difficult to discriminate between the two causes of crop loss, i.e., soil exhaustion or Striga infestation, because soil exhaustion is normally accompanied by a steady increase in Striga when grain sorghum is grown year after year. As an explanation of the situation on the clays and sands of West Sudan, Wilson - Jones suggested the existence of two strains of Striga - a sorghum strain and a millet strain. Apparently the cultural control of Striga in West Sudan is more advanced and less wasteful of land compared to that of East Sudan but whether the cultural system of West Sudan will be adopted in East Sudan would ultimately depend on the availability of cultivable land. Later work by Tarr (1961) in West Sudan reported that nitrogen deficiency may be more important than Striga attack in limiting yields of Pennisetum millet in the sandy soils.

3. South Sudan

As a continuation of the afore-mentioned argument on cultural control practices in West Sudan, Basinki (1955) asked the same questions of Wilson - Jones (1955) as to whether Striga infestation was only serious on 'worn-out' soils or whether the 'wearing out' was due to Striga itself. Basinki observed that in South Sudan while on comparatively lateritic soils Striga was practically absent, Striga caused yield reductions of up to 70% on rich alluvial clays. The reason for this was attributed to the soil nutrient exhaustion on the lateritic soils due largely to erosion of

the uppermost layer where nutrients accumulate under bush fallow. This soil nutrient exhaustion forces the farmer to abandon the field after a period from 3 to 5 yrs before Striga can become serious (Basinki, 1955). In addition, in the areas where these soils occur, good drainage and comparatively high rainfall permit diversified cropping. On the other hand, alluvial clay soils occur in South Sudan in areas of marginal rainfall and marginal drainage. Sorghum which can tolerate both drought and to a lesser extent water logging, is in these areas the only crop of major importance. Its cultivation is concentrated on sites less liable to flooding and on such sites intensive and prolonged monocropping with sorghum leads to increasing Striga infestations, in time causing serious lowering of yields. Where better drained land is more plentiful and shifting cultivation possible, Striga is regarded by the farmer as the main reason for abandoning old fields (usually after 6 to 8 yr). Basinki (1955) concluded that on inherently fertile heavy soils, damage due to Striga reaches the critical level before the appearance of soil exhaustion. In South Sudan, soil erosion is generally not serious on the clay plains, owing to lack of slope but on their margins where slopes are greater, Striga is one of the factors which dictates shifting cultivation.

4. Central Clays of Sudan

Generally in the central clays of Sudan where land is abundant two major systems of cultural management of Striga are practiced: 'Shifting' and 'Harig' cultivation. These two systems are usually practiced at a considerable distance from villages. It is relevant to mention here that an intensive form of monocropping of sorghum is traditionally practiced around villages. Such intensively cultivated lands are known as 'bilad' lands and because of the continuous monocropping of sorghum the 'bilad' lands are heavily infested with Striga.

Shifting cultivation as a cultural control of Striga occurs on clay soils whose vegetation cover is either forest or bushes. Under shifting cultivation for example after the 4th yr of cultivation with sesame in the 4 yr rotation practiced in East Sudan, the infested land is usually abandoned and allowed to return to bush to regain most of its fetrility before it is recultivated. This may take up to 12 yr (Burnett, 1948). Meanwhile, new forest or bushland is cut down and burnt for cultivation. In contrast to shifting cultivation, 'Harig' cultivation is practiced on clay soils whose vegetation cover is grasses. 'Harig' cultivation is more effective in the cultural control of Striga and at the same time less wasteful of land. It is a form of controlled burning where the intense fires clean the ground for cultivation and reduce subsequent weedings. In 'Harig' cultivation the time allowed for regeneration and growth of grasses is roughly proportional to the length of time the land has been cropped.

B. CULTURAL CONTROL OF STRIGA IN CENTRAL SUDAN

Striga control under irrigation assumes many forms depending on the type of cultivation practiced.

1. 'Gerf' or 'River Bank' Cultivation

On the river banks or 'gerf' lands where sorghum is sown, Striga is not a serious problem because of the annual submergence of these lands by flood water for at least 3 months in addition to the annual erosion or deposition of fresh rich silt on these lands. Ecologically this looks like an 'allogenic' system which ensures annual soil fertility or removal of old soil.

2. Flood Cultivation

Sorghum planted in lands subject to natural flood by the overflow or end spill of seasonal rivers typical of the 'Tokar' and 'Gash' deltas of the Sudan does not support much Striga infestation because of the cultural practices of these areas. In the controlled flood cultivation of the river Gash, the flood water is harnessed and is guided by a system of canals and banks on to definite pre-selected areas. In this way it is possible to rotate the land to be watered and in practice, it is not usual to water the same land in the Gash delta more often than once every 3 yr. Thus, the compulsory resting of the land and the addition of fresh silt contributes to the fertility which is reflected in a healthier sorghum crop and low infestations of Striga.

3. Crop Rotations

Research under intensive agriculture as practiced in the Gezira cotton area of Sudan revealed that a close rotation, i.e., a 3-yr rotation of sorghum, lubia (Dolichos lablab), and cotton induced severe Striga infestations (Andrews, 1945). When two successive fallows were introduced into the rotation, the increased fertility which resulted from this change caused the Striga lessened. Fallowing was reported to improve the physical condition of the Gezira soil and its ability to supply nitrogen (Andrews, 1945). Andrews (1945) concluded that the innate resistance of the host is increased by nitrogen.

4. Spacing, Sowing Pattern, and Sowing Method

The effect of spacing, sowing pattern and method was reported by Last (1960b) and Bebawi and Farah (1981a). Increasing the space between sorghum plants resulted in large decreases of Striga infestations with unmanured crops and small decreases with crops given 80 lb N/acre (Last, 1960a). Changing the geometry of planting from the traditional pattern of regular rows (with plants equidistant from each other) into double rows (with plants arranged in row pairs) resulted in a marked increase in Striga infestation (Bebawi and Farah, 1981a). Striga incidence was less when sorghum was planted on ridged seedbeds than on flat seedbeds.

5. Sowing Date

The intensity of Striga attack was found to be intimately correlated with the sowing date of the sorghum crop. Andrews (1945) grew sorghum in the field during the rainy season (June to October) and reported that the earlier the sowing date within this period the greater the Striga attack.

6. Tillage Operations and Grazing

Under conditions prevailing in the Sudan, tillage operations in practice coupled with soil cracking during the dry season would undoubtedly help to inoculate arable soils at varying depths with Striga seed. Striga seed was reported to be distributed in field soil to a depth of at least 38 cm (Andrews, 1945). Grazing of dry plant material of Striga by domesticated animals after the sorghum fields are harvested was found to contribute to dispersion of Striga seed from one field to the other (Bebawi and Elhag, 1983).

7. Intensity of Irrigations

Light irrigation of the sorghum during the normal sowing period was reported to increase the Striga attack whereas heavy irrigation decreased it (Andrews, 1945).

8. N-fertilization

The value of fertilizer nitrogen as a cultural control measure against Striga depends on the sorghum variety used. Last (1960a) reported that nitrogenous fertilizer given to sorghum variety 'Dibaikri' never decreased the amount of Striga significantly and often increased it whereas with the varieties 'Feterita', 'Dwarf white milo' and 'Wad Fahl' the incidence of Striga was decreased. Bebawi (1981b) found no consistent evidence to indicate that host response to nitrogen fertilization would depress Striga infestations. However, Crowther (1942) indicated that Striga attack may be reduced by the use of nitrogenous fertilizer.

9. Resistant varieties and cultivar mixtures

The use of the indigenous sorghum variety 'Tetron' which has a broad spectrum resistance to Striga populations was reported to sustain a low infestation of Striga under field conditions compared with the great infestations sustained by the susceptible variety 'Ariana' (Bebawi, 1981a; Bebawi and Abdelaziz, 1983). The cultivation of equiproportional mixtures of a susceptible variety 'Ariana' and a resistant variety 'Tetron' was found to reduce Striga infestations by 31% (Bebawi and Abdelaziz, 1983).

10. Hand-pulling

Continuous hand picking of Striga plants before flowering was found to help rid the soil of the seed, but it is essential that the plant be weeded before flowering since green ovaries could produce viable seed (Andrews, 1948).

11. Catch-cropping

Catch crops (host plants parasitized by Striga) are usually used as fallow or green manuring breaks in the rotation. Catch crops are destroyed after they serve their control function. In the Sudan it was found possible to take a catch crop just prior to planting the main crop so as not to waste the season. The incidence of Striga was greatly

reduced in two successive sorghum crops when Sudan grass [Sorghum sudannense (Piper) Stapf.] was ploughed in after only 5 weeks (Last, 1960b). However, Parker (1965) commented that such a procedure would be worthless if the temperature were not high enough early in the season for Striga germination.

12. Trap-cropping

Trap crops in addition to catch crops aim to reduce the viable seed population as rapidly as possible. Trap crops trick the Striga seed into germination without attaching to the crop. Trap crops need not be destroyed after they serve their control function. One popular trap crop in Sudan is lubia (Dolichos lablab) which was found to stimulate Striga seed. Parker (1965) suggested that very severe infestations of Striga could be brought under control with trap cropping provided no new seed is produced.

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RECENT DEVELOPMENTS IN PHLOEM TRANSPORT RESEARCH. THE PARALLEL BETWEEN THE SITE OF ATTACHMENT OF *CUSCUTA* AND DEVELOPING SEEDS OF LEGUMES

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Abstract. By measuring the release of sugars and amino acids from tissues of a strong sink, some general characteristics of phloem unloading could be studied. Data are presented on phloem unloading at the site of attachment of *Cuscuta europaea* L. and phloem unloading in the seed coat of developing legume seeds.

The stimulating influence of *Cuscuta* on sugar release by cells of stem segments appears sucrose-specific, supporting the idea that the stimulating influence of *Cuscuta* is restricted to the sieve-tube system. Evidence is discussed supporting the view that, at the site of attachment of *Cuscuta*, phloem unloading of amino acids is not different from phloem unloading of sucrose.

Using a new experimental system (the embryo is removed from developing legume seeds), it was shown that phloem unloading in developing seeds has the same characteristics as phloem unloading in a stem part parasitised by *Cuscuta*. In experiments with developing legume seeds, it could be demonstrated that phloem transport into the strong sink is turgor-sensitive. Phloem transport into developing seeds and into *Cuscuta* is discussed in relation to the phenomenon of "phloem bleeding" observed in several plant species after making incisions into sieve tubes.

INTRODUCTION

In a plant parasitised by *Cuscuta*, phloem transport is disturbed dramatically. For example, the sink activity of developing fruits of broad bean can disappear completely in a parasitised host (Wolswinkel, 1974a).

From earlier experiments it has been concluded that, in addition to the very efficient absorption system of the parasite as suggested by the anatomical details (Dörr, 1972), an enhanced unloading rate of the phloem is essential for the transfer of assimilates from host to parasite (Wolswinkel, 1974b, 1978, 1979). The process of enhanced phloem unloading is restricted to the parasitised region (Wolswinkel, 1979, 1982) and it is under metabolic control (Wolswinkel, 1974b, 1978; Wolswinkel and Ammerlaan, 1983b). The question arose whether or not the transfer of assimilates from host to *Cuscuta* is comparable with phloem unloading in other strong sinks. If, in broad bean, developing fruits and *Cuscuta* are present together and have to compete for assimilates, *Cuscuta* is the victor (Wolswinkel, 1974a). However, notwithstanding these differences in competitive power, a strong parallel does exist between the "sink strength" of both sinks.

Starting from our data on phloem unloading at the site of attachment of *Cuscuta*, we have designed experiments to measure unloading of assimilates from the phloem of developing legume seeds. The developing embryo can be removed easily from the maternal tissues of pod and ovule (Wolswinkel and Ammerlaan, 1983a). In pulse-labelling experiments, ^{14}C-labelled products of

photosynthesis arrived in the seed coat before they arrived in the embryo (Patrick and McDonald, 1980; Thorne, 1980). For soybean, data on phloem structure in the seed coat have been published (Thorne, 1981).

In this report, experiments with "empty" ovules will be reported. This experimental system has been introduced in experiments with developing seeds of broad bean (Wolswinkel and Ammerlaan, 1983a), but it has also been used for the study of phloem unloading in developing pea seeds (Wolswinkel et al. 1983). Evidence for turgor-sensitive transport of sucrose and amino acids into developing seeds of pea and broad bean will be discussed. Presumably, a relatively low turgor pressure (ψ_p, pressure potential) at the sink end of the phloem pathway, as influenced by the osmotic potential of the apoplast solution in sink tissues, is also important for the very efficient transport of sucrose and amino acids to the site of attachment of Cuscuta.

In this report, also some adiitional data will be presented on the release of sucrose and amino acids by excised broad bean stem segments parasitised by Cuscuta europaea. Much attention will be given to a discussion of some factors which may determine the "sink strength" of the site of attachment of Cuscuta, in comparison with developing legume seeds.

METHODS AND MATERIALS

Plants of broad bean (Vicia faba L. cv. Witkiem) were grown in the laboratory garden during the summer season as described earlier (Wolswinkel, 1974a; Wolswinkel and Ammerlaan, 1983a) and during the winter season in pots with potting compost in a glasshouse (e.g. experiment of Fig. 3). Seeds of pea (Pisum sativum L. cv. Marzia) were obtained from Nunhems Zaden B.V., Haelen, The Netherlands. Plants were grown in a controlled environment cabinet (Wolswinkel et al., 1983) or in the laboratory garden during the summer season. Cuscuta europaea L. was grown as described earlier (Wolswinkel, 1974a). When a seedling had attached itself to a host (broad bean) and had started luxuriant growth, long thin branches of about 30 cm were cut off from the extensively branching parasite and used for infecting other plants.

In pulse-labelling experiments ^3H- or ^{14}C-labelled compounds were administered via the petiole of the leaf subtending the fruiting node (Wolswinkel and Ammerlaan, 1983a) or via the petiole of a leaf situated some distance above the site of attachment of Cuscuta (Wolswinkel, 1978). In experiments with labelled amino acids, L-valine and L-asparagine were chosen since they are quantitatively very important in the phloem sap of legume species and are readily transferred from xylem to phloem in a largely unmetabolised form (many references presented in Wolswinkel and Ammerlaan, 1983a). Moreover, an amino-acid analogue regularly used in studies on membrane transport, α-aminoisobutyric acid (AIB), was chosen (reference in Wolswinkel et al., 1983).

Stem segments of broad bean (about 4 cm) were used (Wolswinkel and Ammerlaan, 1983b). Normally, they were shaken, at 25°C, in 100 ml Erlenmeyer flasks containing 10-15 ml water, to which $CaSO_4$ had been added to a final concentration of 0.5 mM. In the case of parasitised segments, the haustorial coil was removed prior to incubation in 0.5 mM $CaSO_4$.

Methods for measuring the amount of ^3H- and/or ^{14}C-solutes released by plant parts of present in a plant part at the end of a washout experiment have been described earlier (e.g. Wolswinkel and Ammerlaan, 1983a). Methods

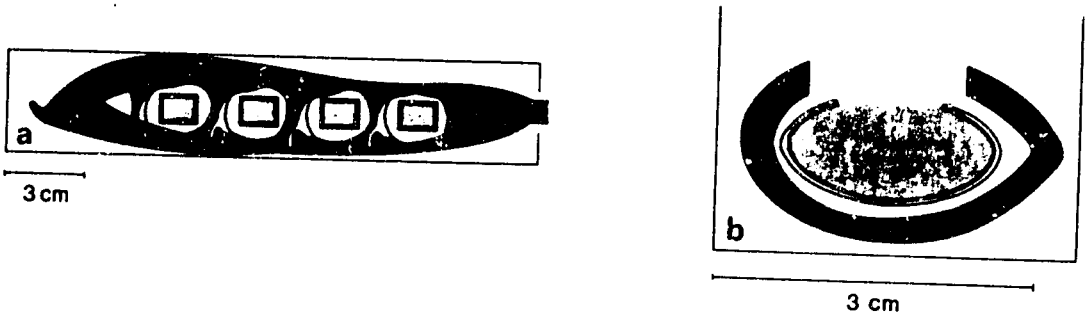


Fig. 1 Diagrammatic representation of the procedure for obtaining "empty" seed coats of Vicia faba and measuring the release of sugar and amino acids by "empty" seed coats. a. The pod is placed in a trough of metal, a window is made in the pod wall and the embryo is removed from four ovules. b. A cross section of one "empty" ovule, filled with a solution (cf. Wolswinkel and Ammerlaan, 1983a).

for enzymatic sugar and amino acid analysis can be found in recent papers (Wolswinkel and Ammerlaan ,1983a,b). Radiochemicals were obtained from the Radiochemical Centre, Amersham, UK (Wolswinkel and Ammerlaan, 1983a).

Prior to measuring transport of sucrose and/or amino acids into developing seeds of broad bean or pea, the embryo was removed from four ovules (Wolswinkel and Ammerlaan, 1983a), as shown in Fig. 1. The part of the seed coat which was excised during the surgical treatment, represented about 10% of the seed coat. The "empty" seed coat was filled with a solution normally containing 0.5 mM $CaSO_4$, 2 mM MES-buffer (pH 5.5), 200 mM sucrose and one or two amino acids (25 mM each). In experiments without isotopes (enzymatic sugar or amino acid analysis), the solution contained 0.5 mM $CaSO_4$, 2 mM MES and 200 mM mannitol. More details can be found elsewhere (Wolswinkel and Ammerlaan, 1983a; Wolswinkel et al., 1983).

RESULTS AND DISCUSSION

1. Sucrose-specific stimulating influence of Cuscuta on sugar release by excised stem segments

In our earlier papers on the effect of Cuscuta on phloem unloading of assimilates, data on the behaviour of ^{14}C-solutes have been presented (after feeding ^{14}C-sucrose to the plant or incorporation of $^{14}CO_2$ into photosynthate; Wolswinkel, 1974b, 1978, 1979). The question can be raised whether Cuscuta has an effect on all cell types in the host stem or only on the cells of the sieve-tube system. Sucrose is the only sugar detected in the sieve-tube sap of Fabaceae (references in Wolswinkel and Ammerlaan, 1983b). In the experiment of Fig. 2, more sucrose was released from parasitised segments than from non-parasitised segments and the differences were greatest during the last hours (period 4), as reported earlier for ^{14}C-solutes and K^+ (Wolswinkel, 1974b, 1978, 1979). Data on glucose and fructose release from the same experiment have been published elsewhere (Fig. 3 in Wolswinkel and Ammerlaan, 1983b). From data on glucose and fructose release and free space invertase activity it has been concluded that the stimulating influence of Cuscuta on sugar release by cells of stem segments is restricted to sucrose, supporting the idea that this effect of Cuscuta is restricted to

the sieve-tube system (Wolswinkel and Ammerlaan, 1983b).

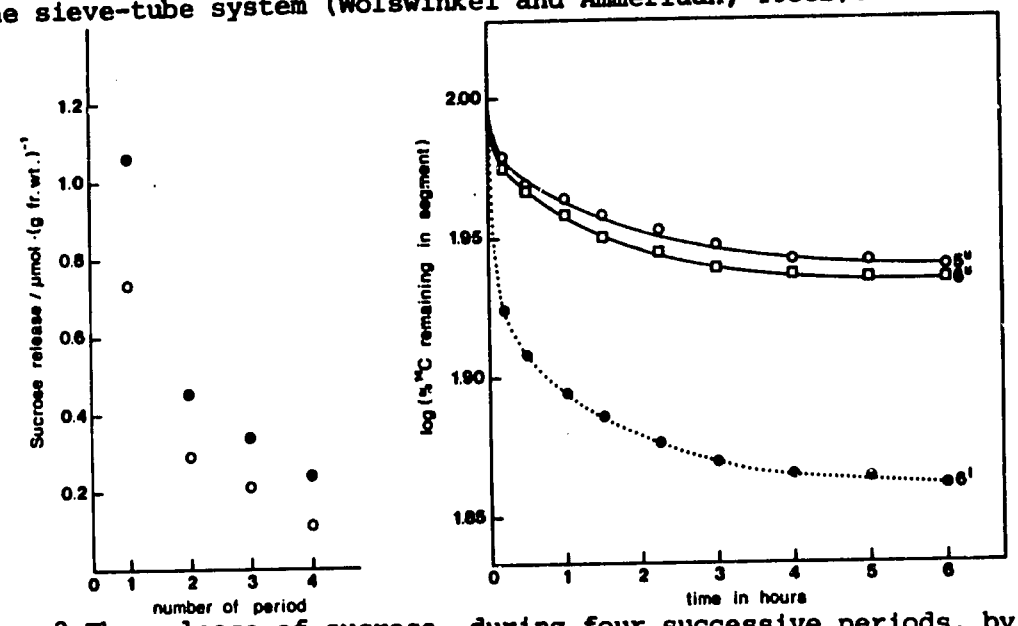
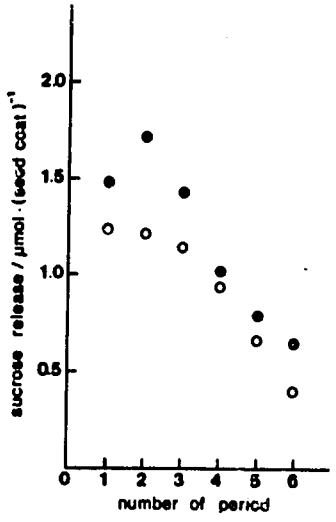


Fig. 2 The release of sucrose, during four successive periods. by parasitised (closed symbols) and non-parasitised (open symbols) segments of five parasitised plants. Data are presented on internode 8 (closed symbols) and on the internodes 7, 9 and 10 (open symbols; the average of the values from the three internodes). The length of the four washing periods was 30 min (period 1), 60 min (period 2), 90 min (period 3) and 120 min (period 4) respectively.

Fig. 3 Effect of Cuscuta europaea on the release of ^{14}C-solutes by excised stem segments, after pulse-labelling with ^{14}C-valine via petiole of leaf 7. Data are presented on the behaviour of a parasitised segment (lower part of internode 6; closed symbols) and two non-parasitised segments (upper part of internode 5, 5^u; upper part of internode 6, 6^u).

2. Evidence for a parallel, at the site of attachment of Cuscuta, between phloem unloading of sucrose and phloem unloading of amino acids

In a previous report, efflux of ^{14}C-amino acids from parasitised segments has been shortly discussed (Wolswinkel, 1979). Especially in some experiments with α-aminoisobutyric (AIB), the pattern of ^{14}C-solute efflux from a parasitised segment was almost similar to the pattern of ^{14}C-solute efflux from non-parasitised segments. In some experiments with L-valine, during the first hours of the experiment the release of ^{14}C-solutes was higher from the parasitised segment than from non-parasitised segments, but it was thought (Wolswinkel, 1979) that the enhanced efflux of labelled valine, during the first 2-3 h of the experiment, represented efflux from the free space and the cytoplasmic compartment of parenchyma cells (cf. Wolswinkel, 1982). Whereas in experiments with labelled sucrose or with K^+ the differences between parasitised and non-parasitised segments were greatest during the last hours of a wash-out experiment (see also Fig. 2), in many experiments with amino acids other results were obtained (e.g. Fig. 3). However, much evidence could be obtained supporting the view that, at



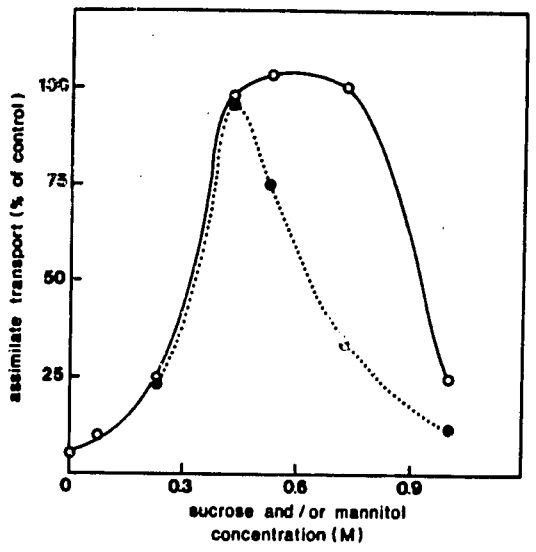


Fig. 4 Effect of mannitol concentration in the solution filling the seed-coat cavity of developing pea seeds on sucrose release into the seed-coat cavity. The total length of the experiment was 12 h (from 10.00 a.m. to 10.00 p.m.). The experiment was carried out in a glass-house, at the end of June. The strong decrease in sucrose release, during the last periods of the experiment (during the end of the afternoon and during the evening), will be the result of lower temperatures at the end of the day. The length of each period was 2 h.

Fig. 5 Optimum-curves for transport of assimilates into ovules (o——o) and release from the seed coat (●---●). Results of pulse-labelling experiments with pea.

the site of attachment of Cuscuta, phloem unloading of amino acids is not different from phloem unloading of sucrose (Wolswinkel et al., 1984). If, after the release of solutes into the apoplast of the stem by phloem unloading, amino acids (and especially AIB) are more intensively taken up by parenchyma cells of the stem than sucrose, then the distribution of labelled amino acids over different compartments (e.g. lumen of sieve elements, cytoplasmic and vacuolar compartments of parenchyma cells; Wolswinkel, 1978, 1979) will be different from that of labelled sucrose. Due to these differences, the effect of Cuscuta on amino acid release (by excised stem segments, several hours after administering labelled solutes via the petiole of a leaf) may be smaller than the effect on sucrose release (Wolswinkel et al., 1984).

3. Parallel between phloem unloading in the seed coat of developing legume seeds and phloem unloading at the site of attachment of Cuscuta

Using the experimental system of Fig. 1 (Wolswinkel and Ammerlaan, 1983a) or using attached or detached seed-coat halves, several authors have recently demonstrated that phloem unloading of sucrose and amino acids from the seed coat is an energy-dependent process, sensitive to metabolic inhibitors. These recent data show a remarkable parallel with the phenomena

observed at the site of attachment of Cuscuta. Data on phloem unloading in parasitised plants have stimulated research on phloem unloading in developing legume seeds (Patrick, 1983; Thorne and Rainbird, 1983; Wolswinkel and Ammerlaan, 1983; Wolswinkel et al., 1983). Physiologically, the presence of Cuscuta seems to imitate the presence of developing seeds. However, Cuscuta is a more powerful sink than developing seeds.

4. Possible significance of data on turgor-sensitive phloem transport into developing legume seeds for the knowledge of transport from host to Cuscuta

In experiments on sucrose and amino acid transport into "empty" ovules of pea and broad bean (using the experimental system of Fig. 1), the osmolarity of the solution filling the seed coat cavity of "empty" ovules appeared to be very important for phloem transport of sucrose and amino acids into "empty" ovules. Data on sucrose release into the seed coat cavity of "empty" pea ovules shown in Fig. 4. A clear difference became visible between sucrose release from seed coats treated with a solution containing 200 mM mannitol (open symbols) and sucrose release from seed coats treated with a solution containing 400 mM mannitol (closed symbols). In parallel experiments with excised "empty" ovules, no clear difference could be found between both treatments (data not shown). From experiments with labelled sucrose and amino acid (Wolswinkel and Ammerlaan, in prep), tentative optimum-curves for transport into an "empty" ovule and release into the solution filling the seed coat cavity (the place where normally, within an intact seed, the embryo is present) can be constructed (Fig. 5). In experiments with about 350 mM (sucrose and/or mannitol) or somewhat higher concentrations, transport of labelled sucrose and amino acid into "empty" ovules was practically similar to transport into intact ovules (the controls being present within the same fruit). Apparently, with respect to an optimal transport of sucrose and amino acids into developing ovules, the unloading sites of the seed coat are characterised by an environment which can be imitated by an apoplast solution containing about 350 mM sucrose or mannitol. The "sink strength" of a developing legume seed seems dependent on a high solute concentration (e.g. sugars, amino acids, mineral ions) in the seed coat apoplast, at least in the region of the unloading sites. Since such a strong parallel does exist between the "sink strength" of developing legume seeds and that of the site of attachment of Cuscuta, this phenomenon may also represent an important factor determining the rate of phloem transport into the site of attachment of Cuscuta.

5. "Sink strength" of the site of attachment of Cuscuta, in comparison with developing legume seeds

Our experiments present additional evidence that phloem transport from source to sink is dependent on turgor-pressure differences between sieve tubes of source regions and sieve tubes of sink regions (see also several chapters in Zimmermann and Milburn, 1975). Addition of sucrose or mannitol to the solution filling the seed coat cavity will change ψ_s (osmotic potential or solute potential; Smith and Milburn, 1980 a,b) of the solution present in the seed coat apoplast. This will cause an osmotic efflux of water from the sieve tubes of the seed coat and a reduction of phloem ψ_p

(pressure potential or turgor pressure; Smith and Milburn, 1980 a,b). The propagation of the change in ψ_p along the sieve-tube pathway can occur very rapidly (Smith and Milburn, 1980a) and recent data suggest that also phloem loading of sucrose can be stimulated by a reduction in phloem ψ_p (Smith and Milburn, 1980b). A high solute concentration in the apoplast of sink tissues (e.g. the site of attachment of Cuscuta and the seed coat of developing legume seeds) may be maintained by metabolically controlled phloem unloading. If phloem transport into the sink region and phloem loading in the source region are stimulated by a high solute concentration in the sink apoplast, some parallel may exist with the phenomenon of "phloem bleeding", after the procedure of making incisions into sieve tubes (cf. Smith and Milburn, 1980 a,b). As can be concluded from the premature exhaustion of the host, after infection by Cuscuta or Orobanche, the reaction on the presence of a parasite will be stronger than the reaction on the presence of developing fruits. However, a strong parallel does exist. If the premature death or exhaustion of legume plants, as a result of infection by Cuscuta or Orobanche, is regarded as "murder", the more gradual transport of assimilates from maternal plant into developing seeds can be seen as "suicide". Presumably, one of the "secrets" of the competitive power of Cuscuta is the fact that the specialised phloem tissues in the haustoria are situated very strategically, viz. adjacent to the sieve tubes in the main vascular bundles of the host plant (cf. Dörr, 1972). As a result of enhanced phloem unloading at the site of attachment of Cuscuta, the haustoria are "bathed" in an elevated sugar and amino acid concentration. As a result of the high solute concentration in the "bath", the whole host plant may be stimulated to transport solutes into the region of that "bath" (turgor-sensitive processes). Phloem unloading and a high solute concentration in the apoplast of the sink end of the phloem pathway will co-operate in maintaining a high "sink strength" at the site of attachment of Cuscuta.

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PHYSIOLOGICAL APPROACH TO THE CHEMICAL CONTROL OF CUSCUTA: EXPERIMENTS WITH ^{14}C-LABELLED HERBICIDES.

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Abstract- Dodder (Cuscuta) is widespread in France, specially in alfalfa, and available control methods are expensive and inefficient. A careful examination of Cuscuta physiology suggests that phloem-mobile herbicides may be able to destroy selectively the parasite attached to its host plant. This hypothesis is supported by the results of experiments carried out with ^{14}C-labelled herbicides. Dicamba and glyphosate were more strongly accumulated by the parasite than was 2,4-DB. Glyphosate in particular showed promise for selective control.

INTRODUCTION

Dodders (Cuscuta spp) are parasitic weeds difficult to control. They cause losses by reducing yield and sometimes also the quality of many important commercial crops. This parasite is spreading rapidly in France, specially in alfalfa (Medicago sativa L.) (Fer 1981 b and 1983 b; Hacquet et al., 1983). At present the two principal methods used to control Cuscuta are application of chlorpropham (CIPC) or chlorthal (DCPA). Chlorpropham kills Cuscuta at the seedling stage, before its attachment to the host plant (Slater et al., 1969; DAWSON et al., 1969). Chlorthal inhibits attachement of Cuscuta seedlings to treated alfalfa (Bayer et al., 1965). These herbicides are not able to control Cuscuta at the parasitic stage, when patches have developed. These available methods seldom give 100 percent control, and effects last only for 3 to 5 weeks after the herbicide application. So, repeated treatments are needed as Cuscuta emergence occurs sporadically throughout the growing period of alfalfa (Koyama and Furuya, 1979; Hutchison and Ashton, 1980). These control methods are not extensively used in France because they are too expensive and not sufficiently effective.

A new control method is therefore needed which will destroy the parasite after its attachment to the host plant, without harming the crop. Such a method would have several important advantages: the herbicide would be applied only on Cuscuta patches, at a stage when the farmers can easily see the parasite and the cost of such treatments could be lower.

To choose, among the available herbicides that may be of interest in

selectively controlling Cuscuta attached to its host plant, it is necessary to summarize the physiological particularities of the parasite.

Cuscuta at the parasitic stage is characterized by a very reduced photosynthetic activity (Fer, 1977). Thus, the parasite must obtain large quantitaties of organic substances from the host plant. It is now well known that organic substances are obtained from the host mainly as sucrose (Jacob and Neumann, 1968; Fer, 1978) and to a lesser extent as amino-acids (Fer, 1981 a). These metabolites are obtained from the host phloem sap. Experiments with ^{14}C-sucrose have shown that more than 80 % of the sucrose exported out of a parasitised leaf was taken up by Cuscuta (Jacob and Neumann, 1968; Wolswinkel, 1977; Fer, 1979). Mineral ions such as K^+ and $PO4^{=}$) are also derived from the host phloem. On the contrary very low amounts of Ca^{++} (a typical xylem-mobile ion) are derived by Cuscuta from the host xylem sap (Fer, 1981 a); this last result suggests a very low transpiration rate of the parasite. The transpiration rate of this parasite has often been supposed to be low (Tsivion, 1978; Schaffner, 1979), but direct measurements have never been performed. Results reported below confirm such a low transpiration rate in Cuscuta and this suggests that systemic herbicides that are translocated in the host xylem via the transpiration stream must probably be discarded. Indeed, one may expect that such herbicides would move and accumulate mostly in the host organs with high transpiration rate and would be more deleterious for host than for parasite.

On the contrary, one may expect that systemic herbicides that are mobile in the host phloem pathway will be taken up and accumulated by the parasite. The deleterious effects of such herbicides would be greater for the parasite than for the host plant. In order to test this hypothesis experiments have been carried out with three labelled herbicides differing in their phloem mobility: 2,4-DB, a poorly phloem-mobile herbicide, dicamba and glyphosate, two highly phloem-mobile herbicides (Ashton and Crafts, 1973; Christ, 1979).

METHODS AND MATERIALS

1. Plant material.

Cuscuta species used were C. lupuliformis Krock. and C. reflexa Roxb. and host species were Pelargonium zonale (L.) Aiton and Ricinus communis L.

The experiments with labelled herbicides were carried out using isolated host leaves parasitised by a young growing Cuscuta plant (Fig.1); this model has been used previously for transport studies (Fer, 1981 a).

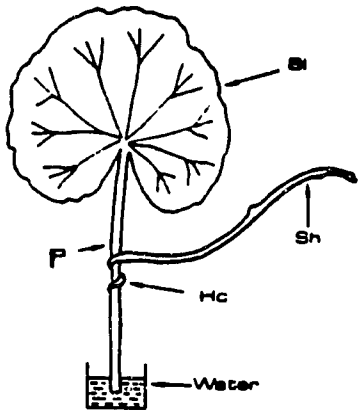

Figure 1.

Plant material used: detached Pelargonium leaf parasitised by a young Cuscuta plant.
- Bl : leaf blade.
- P : leaf petiole.
- Hc : Cuscuta haustorial coil.
- Sh : growing young Cuscuta shoot.

2. Herbicides used and modes of application

Three ^{14}C-Labelled phloem-mobile herbicides were used: 2,4-DB-1-^{14}C, dicamba ring-^{14}C (U) and glyphosate methyl-14C. These herbicides were applied as pure active substances dissolved in distilled water without addition of surfactant or other formulation products. The amount of herbicide applied is specified for each experiment. The herbicide treatments were performed by standard laboratory methods (Fer and Chamel, 1983).

In most cases, the herbicide solution was applied on the upper side of the host leaf blade (as a droplet or as a spray). In some cases the herbicide solution was quickly absorbed through the cut end of the isolated leaf petiole which was then fed with distilled water. The absorbed herbicide was translocated into the host leaf blade by the transpiration stream. The treated parasitised leaves were then kept in a growth chamber (Fer and Chamel, 1983) or in the laboratory for 1 to 5 days.

On Cuscuta the herbicide solution was applied as a droplet on the surface of the shoot.

Each experiment was replicated at least three times.

3. Radioactivity localization and measurement.

At the end of the experimental period leaves which had received the labelled herbicide on the blade (droplet or spray) were washed to remove the non absorbed herbicide. The radioactivity of the washing solutions was measured by scintillation counting (Packard spectrometer). The parasitised leaves were divided into several parts: leaf blade, leaf petiole, Cuscuta haustorial coil and Cuscuta shoot, before drying in an oven at 50°C. The separation of the parasitised leaves into several parts was to prevent

movement of the herbicide between these different parts during the drying process. Autoradiographs were obtained by application of dried plant material against kodak radioagraphic films.

The radioactivity measurements were performed after combustion of the samples in a Packard Oxidizer. The labelled CO_2 evolved during the combustion process was absorbed by carbosorb (Packard) and the radioactivity was measured using permafluor V (Packard) as the scintillation coktail.

RESULTS

1. Transpiration of Cuscuta

An experiment was carried out using castor bean (*Ricinus communis* L.) growing in pots and parasitised by *C. lupuliformis*. The pots enclosed in polyethylene bags to prevent water loss from the soil, were placed on the balance. The water loss of the aerial part of the parasitised plants was measured each 30 mn for 2 h. *Cuscuta* shoots were then harvested and the water loss of the host plant alone measured (each 30 mn for 2 h). This experiment was carried out in July, between 10 am and 2 pm; under direct sunlight; temperature was 26°C and relative humidity 65 %.

The results (table 1) show that transpiration of *C. lupuliformis*, expressed on a fresh weight basis, was only 2 % of that of the host leaf transpiration rate. So, it is clear that the total amount of water derived from the host plant by the parasite is very small. Moreover, experiments conducted with tritiated water (not reported here in detail) show that a part of the water derived from the host is obtained by *Cuscuta* from the host plant phloem pathway. So, it can be concluded that the amount of water derived by *Cuscuta* from the xylem of the host plant may be very low.

Table 1

Transpiration rates of Cuscuta and host leaves.

Plant material	Transpiration rates mg $H_2O.g^{-1}$ fresh weight$.h^{-1}$
Ricinus communis leaves (blades only)	369 ± 41
C. lupuliformis shoots growing on *Ricinus*	7.5 ± 1

Values= mean of 4 replications ± standard error of the mean

2. Experiment with 2,4-DB

This experiment was conducted using three isolated Pelargonium leaves parasitised by C. lupuliformis. The labelled 2,4-DB solution was applied as a droplet (100 µl = 5 µg of 2,4-DB) on the upper side of the host leaf. Five days after the treatment 30 to 50 per cent of the applied 2,4-DB had been absorbed by the leaf blade. Autoradiographs of the treated parasitised leaf, showing the precise localization of the absorbed herbicide are presented elsewhere (Fer and Chamel, 1983). The distribution of the absorbed 2,4-DB in the different parts of the leaf and in the parasite is given in table 2. These results provide evidence that 15 per cent of the 2,4-DB absorbed by the host leaf was transfered to the parasite. According to the mode of application of the 2,4-DB solution, it can be concluded that the transport of this herbicide from host to parasite takes place through the phloem pathway (Fer, 1981 a). At the end of the experimental period no phytotoxic effects were observed, either on host leaf nor on parasite.

Table 2

Distribution of the absorbed 2,4-DB in the different parts of the leaf and in the parasite 5 days after application as a droplet on the host leaf blade.

Plant part	2,4-DB content, expressed as % of the total amount absorbed by the leaf blade
Treated area of the leaf blade	72.5 ± 5.6
Other part of the leaf blade	9.7 ± 1.9
Leaf petiole with attached Cuscuta haustorial coil	2.3 ± 0.7
Cuscuta shoot	15.5 ± 3.2

Values = mean of 3 replications ± standard error of the mean.

3. Experiment with dicamba

The experiment reported here was carried out with four isolated Pelargonium leaves parasitised by C. lupuliformis. The labelled dicamba solution was applied as a spray (1 ± 0.2 ml = 14.4 ± 2.9 µg of dicamba) on the blade of each leaf. Four days after the treatment the leaf blades had absorbed 86 to 90 % of the applied dicamba. The distribution of the absorbed herbicide in the different parts of the leaf and in the parasite (table 3) shows that 72.5 % of the absorbed dicamba was exported, via the phloem pathway, into the parasite. Moreover the dicamba concentration in the parasite shoot (220.8 $\mu g.g^{-1}$ of dry weight) was about thirtyfold higher than in the treated host leaf blade (7.24 $\mu g.g^{-1}$ of dry weight). An

autoradiograph showing dicamba localizaton in the treated parasitised leaf is presented in another paper (Fer and Chamel, 1983).

Table 3

Distribution of the absorbed dicamba in the different parts of the leaf and in the parasite 4 days after application as a spray on the host leaf blade.

Plant part		Dicamba content µg	Dry weight of Plant part mg	Dicamba concentration µg/g. of Dry weight
Host leaf	blade	2.60 ± 0.22 (22.09)*	359 ± 20	7.24 ± 0.42
	petiole	0.64 ± 0.14 (5.43)*	89 ± 9	7.19 ± 1.70
Cuscuta	haustorial coil	0.58 ± 0.10 (4.92)*	6 ± 1	96.6 ± 8.4
	shoot	7.95 ± 0.71 (67.54)*	36 ± 3	220.8 ± 17

Values = mean of 4 replications ± standard error of the mean.
* Values in parenthese are % of the total dicamba absorbed by the host.

At the end of the experimental period, phytotoxic effects were observed on the Cuscuta: the oldest part of the shoot had coiled itself into spiral; the shoot was bent at the nodes; the apical part of the shoot had stopped its circumnutation movement and the growth rate of the shoot was lower, but growth was not stopped.

4. Application of glyphosate to the host leaf.

a) The herbicide applied as a droplet on the upper side of the host leaf blade. This experiment was conducted using 6 isolated Pelargonium leaves parasitised by C. lupuliformis. The labelled glyphosate solution was applied as a droplet (10 µl = 8.8 µg of glyphosate) on the upper side of each host leaf blade. Four days after the treatment the leaf blades had absorbed 24 to 43 % of the applied glyphosate (mean = 32.7 %). Autoradiographs (not presented) of the treated leaves showed that the absorbed herbicide had been accumulated by the parasite; Moreover it was observed that the labelled herbicide had not distributed uniformly within the parasite: glyphosate accumulated much more in the apical part of the Cuscuta shoot (especially in buds) than in the haustorial coil.

The distribution of the absorbed herbicide in the different parts of the leaf and in the parasite (table 4) showed that 75 % of the absorbed glyphosate had been translocated via the phloem pathway into the parasite.

The glyphosate concentration was seventeen fold higher in the parasite (47.4 $\mu g.g^{-1}$ of dry weight) than in the treated host leaf blade (2.79 $\mu g.g^{-1}$ of dry weight).

Table 4

Distribution of the absorbed glyphosate in the different parts of the leaf and in the parasite 4 days after application as a droplet on the host leaf blade.

Plant part	glyphosate content (μg)	Dry weight of plant part (mg)	Glyphosate concentration $\mu g.g^{-1}$dry weight
Leaf blade	0.58 ± 0.06 (20.0)*	212 ± 15	2.79 ± 0.29
Leaf petiole	0.14 ± 0.04 (4.8)*	75 ± 4.5	1.80 ± 0.51
Dodder	2.18 ± 0.19 (75.2)*	50 ± 9.5	47.4 ± 9.1

Values = mean of 6 replications ± standard error of the mean.
* Values in parentheses are % of total glyphosate absorbed by the host.

At the end of the experimental period, the following phytotoxic effects were observed on the Cuscuta: the oldest part of the shoot had coiled itself into spiral; the shoot, usually red in this species, became rose-coloured; the growth and the movements of shoots were strongly inhibited; in some cases, necrosis was observed on buds.

Table 5

Distribution of the absorbed glyphosate in the different parts of the leaf and in the parasite 4 days after glyphosate was introduced, through the cut end of the petiole, in the host leaf transpiration stream.

Plant part	Glyphosate content (μg)	Dry weight of plant part (mg)	Glyphosate concentration $\mu g.g^{-1}$.dry weight
Leaf blade	1.365 ± 0.100 (33.69)*	319 ± 18	4.10 ± 0.45
Leaf petiole	0.826 ± 0.095 (20.39)*	87 ± 4	9.5 ± 1.1
Cuscuta	1.860 ± 0.292 (45.92)*	33 ± 7	65.6 ± 5.8

Values = mean of 8 replications ± standard error of the mean.
* Values in parentheses are % of the total glyphosate absorbed by the host.

b) The herbicide introduced in the host leaf transpiration stream. This experiment was carried out using 8 isolated Pelargonium leaves parasitised by C. lupuliformis. Each leaf absorbed through the cut end of its petiole a small volume of labelled herbicide solution (50 µl = 4.4 µg of glyphosate) and then was fed with distilled water. Four days after the treatment, about 46 % of the applied glyphosate had been exported via the host phloem pathway into the parasite (table 5). Moreover the glyphosate concentration was sixteenfold higher in the parasite (65.6 $\mu g.g^{-1}$ of dry weight) than in the host leaf blade (4.1 $\mu g.g^{-1}$ of dry weight). At the end of the experimental period the phytotoxic effects previously described were also observed.

5. Application of glyphosate to the Cuscuta shoot.

This experiment was carried out using C. reflexa growing on R. communis. Four terminal Cuscuta shoots were selected; they were 30 to 40 cm long at the beginning of the experiment. A droplet of the herbicide solution (30 µl = 26.4 µg of glyphosate) was applied on the middle part of each shoot (fig. 2). Twenty hours after the treatment each shoot had grown 10 to 15 cm, a normal growth rate for this species. The shoots were then harvested and divided into 3 cm long segments and the radioactivity of each segment measured.

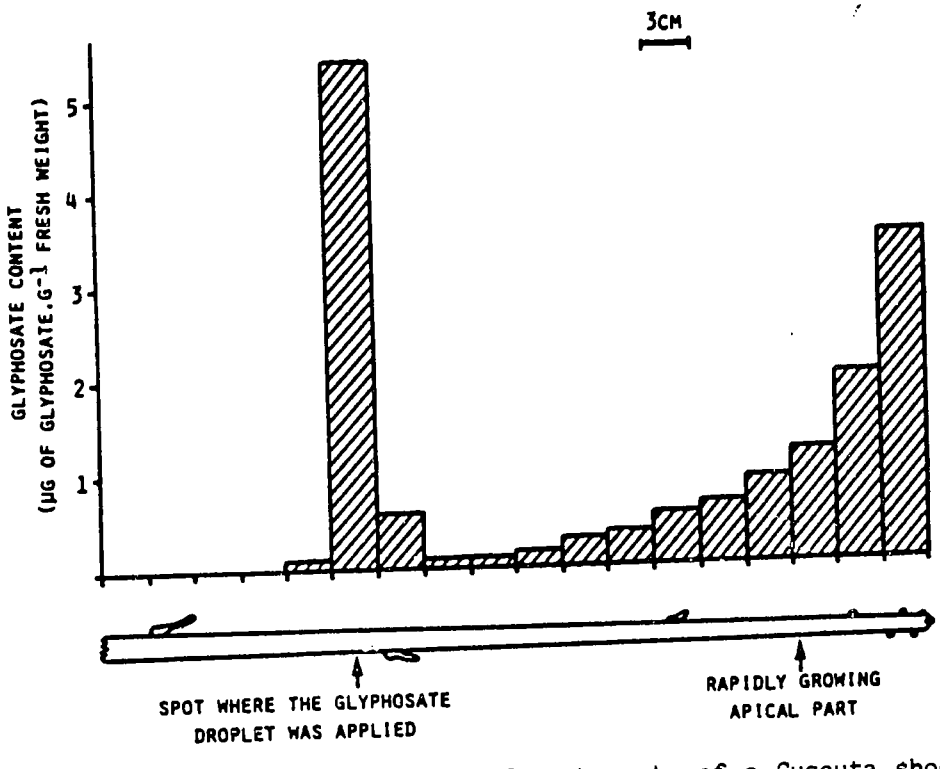

Figure 2.- Glyphosate content of the different parts of a Cuscuta shoot 20 h after applying a droplet of ^{14}C-glyphosate solution on the middle part of the shoot. The Cuscuta shoot is drawn below the absciss line.

Each Cuscuta shoot absorbed 12 to 17 % of the applied glyphosate. About 50 % of the absorbed herbicide moved out of the treated part of the shoot, but only in the upward direction. The translocated glyphosate accumulated in the apical region of the Cuscuta shoot which had grown during the experiment. Figure 2 reports the results obtained with one of the C. reflexa shoots used.

DISCUSSION

Transpiration rate was demonstrated to be very low in C. lupuliformis. This and the data on nutrition of the parasite show that highly phloem-mobile herbicides may be of interest in Cuscuta control. This hypothesis was tested using three labelled herbicides.

The isolated parasitised leaf was proved to be a good miniature model of the host-parasite system. This model possesses all the components required for studies relative to the transport of phloem-mobile substances: a source (the leaf blade), a long distance transport pathway (the leaf petiole) and a sink (the attached parasite).

Because of the small size of the model it was possible to carry out many experiments with limited amounts of labelled herbicides, the cost of which is often very high. Nevertheless the isolated parasitised leaves can be preserved in a good physiological state for a period of 7 to 8 days only; the duration of the experiments must therefore be limited to 4 or 5 days.

Experiments with 2,4-DB, dicamba and glyphosate demonstrated that these three herbicides were exported out of the treated host leaf blade, via the phloem pathway, and were taken up by Cuscuta. The ability of the parasite to derive the natural substances (sucrose, amino-acids, mineral ions...) moving along the host phloem pathway had previously been well demonstrated (Jacob and Neumann, 1968; Wolswinkel, 1977; Fer, 1978, 1979 and 1981 a). It is now shown that Cuscuta is also able to take up xenobiotic substances moving in the host phloem.

These three phloem-mobile herbicides are taken up by Cuscuta but they are not accumulated to the same extent in the parasite. Dicamba and glyphosate (two highly phloem-mobile herbicides) are much more accumulated in Cuscuta than 2,4-DB (a poorly phloem-mobile herbicide). So, the ability of Cuscuta to take up and to concentrate herbicides applied to the host leaves depends on the mobility level of these herbicides in the host phloem pathway.

If we consider now the distribution of the absorbed herbicides within the parasite, differences can be observed according to each herbicide. In

the case of 2,4-DB and dicamba, the absorbed herbicide is almost uniformly distributed along the parasite shoot (Fer and Chamel, 1983). In the case of glyphosate, the absorbed herbicide is accumulated much more in the meristems (vegetative and floral buds) than in the other parts of the shoot.

Phytotoxic effects could be observed on Cuscuta 2 to 4 days after the application of dicamba or glyphosate on the host leaves.

Cuscuta not only derives large quantities of glyphosate from the host plant but also absorbs glyphosate applied directly on its shoots. So, in field conditions, after spraying the host and the attached parasite, the amount of glyphosate taken up by the parasite would be higher than in our experiments.

The absorbed glyphosate moves within the parasite only in the upward direction. The unidirectional translocation, within the parasite, of the assimilates derived from the host has previously been demonstrated (Fer, 1977). So, a xenobiotic substance such as glyphosate is translocated in the host-parasite system in the same way as the assimilates.

All the experiments reported in this paper strongly suggest that selective destruction of the attached parasite using low rates of highly phloem-mobile herbicides may be a promising method of Cuscuta control. In fact, since this new strategy of control has been envisaged (Fer, 1981 b), it has also been tested at the field level and the preliminary results are encouraging (Dawson and Saghir, 1982 and 1983; Hacquet et al., 1983). This new strategy must therefore be further investigated, at laboratory and field levels, to determine with precision the conditions required for total and selective destruction of the attached Cuscuta in alfalfa, and in other important commercial crops.

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BIOCHEMICAL ASPECTS OF PARASITISM IN CUSCUTA REFLEXA : INHIBITION OF CELL WALL DEGRADING ENZYMES OF CUSCUTA BY NON-SUSCEPTIBLE PLANTS

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Abstract Cell-wall degrading enzymes were earlier demonstrated by us in *Cuscuta reflexa* Roxb. and its hosts. To explore the mechanism of parasitism, studies were made to test the factors affecting the activities of cell wall degrading enzymes in susceptible and non-susceptible plants. Pectin methylesterase, polygalacturonase, cellulase-Cl and cellulase-Cx activities of *Cuscuta* were inhibited by extracts of *Ipomoea batatas* (L.) Lam. and *Solanum tuberosum* L. suggesting the presence of inhibitor(s) of these enzymes in *Ipomoea* and *Solanum*. The inhibitor(s) of *Cuscuta* cellulase-Cx from *S. tuberosum* and *I. batatas* was characterised. The inhibitor(s) was water soluble, heat resistant (100°C, 5 min) and low molecular weight dialysable substance(s). Dixon plot indicated a non-competitive nature for the *Cuscuta* cellulase-Cx inhibitor. The inhibitor action could be abolished on addition of EDTA suggesting a metallic nature of the inhibitor(s). Of the divalent ions tested, Ca^{++} proved to be the most potent inhibitor of *Cuscuta* cellulase-Cx followed by Mg^{++}, Mn^{++} and Fe^{++}. Five to 10 fold higher content of calcium was found in non-susceptible plants compared to susceptible hosts. The inhibitor(s) of the parasite cellulase-Cx activity present in non-susceptible plants appears to be Ca^{++} either alone or in combination with Mg^{++}, Mn^{++} and Fe^{++}. In preliminary greenhouse experiments spraying the infected plants with cellulase inhibitor, Ca^{++}, led to selective destruction of the parasite.

INTRODUCTION

Krohn (1984) reported that not all plants are susceptible to parasitism from dodder. Gaertner (1950) has listed species immune to dodder. Very little has been done so far to investigate physiological and biochemical aspects of host specificity of parasitic plants (Tsivion, 1978a). Based on the application of cytokinin in haustorial development, Tsivion (1978b) concluded that *in vivo* the recognition of plants by *Cuscuta* exhibits little or no specificity. Where specificity for a plant host does exist, it is not connected with the level of recognition. It is, therefore, of interest to explore other factors responsible for making some plants non-susceptible to parasitism for dodder. To examine if the non-susceptibility of certain plants is due to the presence of the inhibitor(s) of key

enzymes involved in haustorial development, experiments have been conducted on Solanum tuberosum L. and Ipomoea batatas(L.) Lam. which are non-susceptible to parasitism by Cuscuta reflexa and results are reported below.

METHODS AND MATERIALS

Plants

Alfalfa (Medicago sativa L.), bean (Phaseolus vulgaris L.), lantana (Lantana camara L.), makoi (Solanum nigrum L.), mustard (Brassica campestris L.), pea (Pisum sativum L.) and sunflower (Helianthus annuus L.) were used as hosts for Cuscuta reflexa Roxb. In addition to the susceptible hosts, sweet potato (Ipomoea batatas) and potato (Solanum tuberosum), which are not susceptible to dodder infection, were grown in the departmental garden under natural conditions of light and humidity. From plants, about 2 months old, 6 cm twigs were cut from vigorously growing branches and leaf blades removed. The resulting stems along with petioles were used in the following experiments.

Tissue homogenisation and enzyme extraction

Pectinmethyl esterase (PME), polygalacturonase (PG), xylanase, cellulase-Cl (active against natural cellulose) and cellobiase enzymes were extracted by the method of Ahmed and Labavitch (1980). Cellulase-Cx (active against CM-cellulose) was extracted by the method of Pharr and Dickinson (1973).

Preparation of inhibitor extract

A 20 % (w/v) homogenate of I.batatas/S. tuberosum was prepared by grinding 4 g of tissues (stems and petioles) with 20 ml of 0.1 M Na-acetate buffer, pH 5.0 in a chilled pestle and mortar. The homogenate was centrifuged at 15,000 x g for 30 min and the supernatant obtained was entitled the inhibitor extract. In some experiments the inhibitor extract was dialysed in a cellophane bag with several changes of water for 24 h. at 0-4°C. A total dialysate volume of 500 ml was obtained from 10 ml of the supernatant. The dialysate was concentrated in a flash evaporator at 40°C to 10 ml.

Enzyme assays

All enzyme assays were carried out under conditions of linearity with respect to enzyme concentration and time and at optimum substrate concentration. The PME activity was determined according to the method of Kertesz (1955). PG, xylanase and cellulase-Cl activities were determined by the procedure adopted by Ahmed and Labavitch (1980). The cellu-

lase-Cx activity was measured according to the method of Pharr and Dickinson (1973). One unit (U) of cell wall hydrolytic activity is defined as the amount of enzyme required to catalyse the release of 1 u mole of glucose per min. One unit of PME caused the hydrolysis of one equivalent of ester in one min.

The inhibitory effect of extracts on cell wall degrading enzymes of Cuscuta was determined by adding different amounts of extracts in the assay system in place of water.

Estimation of calcium

The calcium content was determined according to the method of Humphries (1956).

RESULTS

Search for factors affecting the activities of cell wall degrading enzymes of the parasite in susceptible and non-susceptible plants

To test the presence of inhibitor(s) or activator(s) of cell wall degrading enzymes of the parasite in host and non-host plants, equal amounts (0.5 ml of 20% w/v homogenate) of extracts from C.reflexa and host/non-host plants were mixed and activities determined in the mixed extract and individual extracts. The sum of activities of individual extracts were higher than activities in the mixed extracts for PME, PG, cellulase-Cl and cellulase-Cx for non-susceptible plants. I.batatas and S.tuberosum (Table 1) suggesting the presence of an inhibitor(s) of these enzymes. No such inhibition was observed for xylanase activity of the parasite. On mixing the extracts of susceptible host, H.annuus or M.sativa and Cuscuta, no inhibition of cell wall degrading enzymes of Cuscuta was observed.

Table 1

Effect of mixing of extracts of non-susceptible plants and Cuscuta on cell wall degrading enzymes of Cuscuta

	Enzyme activity, mU				
	PME	PG	Xylanase	Cellulase-Cl	Cellulase-Cx
Cuscuta	59	3.8	3.5	2.4	0.65
I.batatas	19	2.0	3.9	0	0.20
Cuscuta + I.batatas	28	1.2	7.6	1.9	0.09

Inhibition (%)	64	80	0	21	90
Cuscuta	58	3.8	3.5	2.4	0.64
S. tuberosum	28	1.5	1.2	0	0.20
Cuscuta + S. tuberosum	34	0.9	4.7	1.9	0.08
Inhibition (%)	60	83	0	20	90

Characterisation of inhibitor(s) of cellulase-Cx

Effects of addition of 0.2 ml of differently treated Ipomoea/Solanum extracts to 0.5 ml of Cuscuta cellulase-Cx enzyme were studied (Table 2). Heating the inhibitory extracts from Ipomoea and Solanum in a boiling water bath for 5 min did not lead to any loss in the inhibitory activity. Dialysis against water resulted in major loss of the inhibitory activity. The inhibitor activity was recovered in the dialysate of heated extracts. Dialysis or desalting of the inhibitor extract led to slight enhancement of cellulase-Cx activity of Ipomoea/Solanum.

Table 2

Effect of addition of Ipomoea and Solanum extracts on Cuscuta cellulase-Cx activity

Additive	Ipomoea			Solanum		
	1 mU	2 mU	Inhibition %	1 mU	2 mU	Inhibition %
Nil		0.62			0.60	
Untreated extract	0.07	0.11	84	0.08	0.08	88
Dialysed extract	0.09	0.66	7	0.10	0.65	7
Heat treated extract	0	0.07	89	0	0.06	90
Dialysed heated extract	0	0.58	7	0	0.56	7
Dialysate from heated extract	0	0.08	87	0	0.07	88

1-Enzyme activity; 2-Observed mixed activity with Cuscuta.

Kinetics of inhibition

The percent inhibition of cellulase-Cx activity was found to increase on increasing the amount of the inhibitor(heated dialysate of S.tuberosum), tested at two concentrations of the substrate. The Dixon plot of the data (Fig.1) shows no change in the Km value for Cuscuta cellulase-Cx in the presence of the inhibitor, but the Vmax value was decreased. This suggests non-competitive nature of the inhibitor.

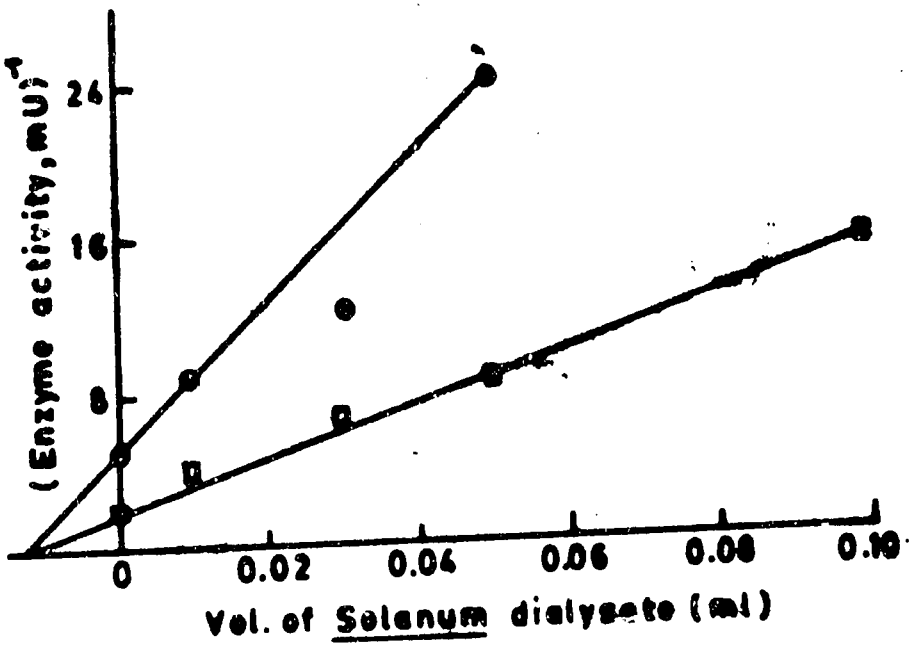

Fig.1 Dixon plot for inhibition of Cuscuta cellulase-Cx activity by heat treated dialysate from S. tuberosum. ⊙——⊙, 0.23 % (w/v) CM cellulose; □——□, 0.7 % (w/v) CM cellulose.

Effect of EDTA and metal ions on Cuscuta cellulase activity

1 and 10 mM EDTA did not produce any effect on Cuscuta cellulase-Cx activity. The addition of 0.2 ml of heated dialysate from Solanum extract to 0.5 ml of Cuscuta cellulase-Cx enzyme led to about 90 % inhibition of the enzyme activity, which was reversed on addition of the metal chelating agent, EDTA (Table 3). This suggests metallic nature of cellulase-Cx inhibitor present in the dialysate of Solanum extract. Of the various metal ions tested, Ca^{++} was the potent inhibitor of Cuscuta cellulase-Cx followed by Mg^{++}, Mn^{++} and Fe^{++}.

Table 3

Reversal of Cuscuta cellulase-Cx inhibition by EDTA and effect of metal ions

Additive	Activity mU	Inhibition %
Control	0.65	
Control + heated dialysate from *Solanum*	0.06	91
---------- + EDTA (1 mM)	0.54	17
---------- + EDTA (10 mM)	0.60	8
Control + $CaSO_4$ (1 mM)	0.25	62
" + $MgSO_4$ (1 mM)	0.36	45
" + $MnSO_4$ (1 mM)	0.41	37
" + $FeSO_4$ (1 mM)	0.54	17

Comparison of calcium content of stems and petioles of non-susceptible and susceptible hosts of Cuscuta

Calcium content of *I.batatas* and *S.tuberosum*, which are not susceptible to parasitism by *Cuscuta*, was significantly higher than the susceptible hosts (Table 4). Amongst susceptible hosts, Ca^{++} contents of *P.vulgaris* and *S. nigrum* were higher compared to other hosts.

Table 4

Calcium content of petioles and stems of non-susceptible and susceptible hosts of Cuscuta

Plants	% calcium content	
	Fresh weight basis	Dry weight basis
Susceptible :		
B.campestris	0.019	0.18
H.annuus	0.021	0.15
L.camara	0.021	0.13
M.sativa	0.018	0.07
P.sativum	0.021	0.19
P.vulgaris	0.041	0.26
S.nigrum	0.030	0.26

Non-susceptible :		
I.batatas	0.15	1.3
S.tuberosum	0.20	1.7

The values reported are mean values of 3 experiments.

Control of Cuscuta by Calcium

To test if calcium has any effect on the survival of Cuscuta, several garden experiments were carried out. S.nigrum plants (about 50 cm in height and about 2 months old), grown on pots in garden soil, were infected with the parasite by twining 15 cm length of Cuscuta filaments. In about 7-10 days, Cuscuta was well established and vigorous infection had occurred. At this stage, 6 plants each of S.nigrum were sprayed till thorough wetting with various concentrations of $CaCl_2$. About 25 ml of $CaCl_2$ solution was used per plant. Spraying with 0.5, 0.75 and 1 M concentrations of $CaCl_2$ regularly for 5 days (once every day) led to browning of Cuscuta and wilting of the host plant with leaves turning to pale brown. Spraying with 0.25 M $CaCl_2$ for 5 days destroyed Cuscuta but leaves of the host plant turned yellow and wilted. The wilted host plants on watering for 20 days resulted in greening of leaves with recovery to original state. Successful eradication of the parasite was achieved on spraying with 0.1 M $CaCl_2$ once a week for five weeks. This resulted in drying of Cuscuta with only slight wilting of the host leaves. Continuous watering for 20 days turned the host plant green and completely healthy.

Similar results were obtained by spraying 0.1 M $CaCl_2$ on Cuscuta parasitising B. campestris.

DISCUSSION

S. tuberosum and I. batatas do not allow haustorial formation and thus no vascular contact with the parasite is made. These plants apparently have a unique biochemical shield which protects against angiosperm parasite. This shield may be composed of a variable array of the so-called secondary substances or internal factor(s) or metabolites which provide resistance to parasites. Metal ions, proteins, phenols, lignin, phytoalexins, plant hormones and stilbenes are variously reported to give resistance to plants against infection by pathogens.

Mixing of extracts either of S.tuberosum or I.batatas with that from C.reflexa resulted in inhibition of pectin methyl esterase, polygalacturonase, cellulase-Cl and cellulase-Cx activities of the parasite. No such inhibition of Cuscuta enzymes was observed on mixing the extracts from susceptible hosts. Thus a characteristic of the non-susceptible plants is

the presence of inhibitor(s) of certain cell wall degrading enzymes of the parasite. The inhibitory action could be abolished on addition of EDTA suggesting a metallic nature of the inhibitor(s). Of the divalent metal ions tested, Ca^{++} proved to be the most potent inhibitor of Cuscuta cellulase-Cx followed by Mg^{++} and Mn^{++}. It was interesting to find over 5-10 fold higher content of calcium in non-susceptible plants compared to susceptible hosts. It thus appears that the inhibitory factor(s) present in non-susceptible plants could be Ca^{++} either alone or in combination with Mg^{++}, Mn^{++} and Fe^{++}. Wallace et al. (1978) reported that % calcium content of leaves of infected hosts of Cuscuta nevadensis ranged from near 1 to over 10, on a dry weight basis.

If C. reflexa cellulase-Cx is involved in haustorial intrusion, the treatment with cellulase inhibitor should prevent haustorial attachment and selectively destroy the parasite. In preliminary greenhouse experiments spraying the infected plants with cellulase inhibitor, Ca^{++}, led to selective destruction of the parasite. Field experiments are to be carried out to assess the efficacy of calcium spray on eradication of Cuscuta. In addition to acting as a Cuscuta cellulase inhibitor, Ca^{++} is very immobile in the phloem (Pate, 1975) and may interfere in the transport of carbohydrate and other material through phloem. Mohanty and Reddy (1982) recently reported reduced susceptibility of rice plant to infection by bacteria Xanthomonas campestris Pv. oryzae on increasing the supply of calcium in the soil.

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A VEGETATIVE CHARACTER THAT SEPARATES SPECIES OF CUSCUTA

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Abstract. A consistent vegetative characteristic separates *Cuscuta planiflora* from *C. campestris* and *C. indecora*. A tendril arises at each node of *C. campestris* and *C. indecora* and twines about the host plant. The primary and lateral stems never twine. In contrast, *C. planiflora* does not bear tendrils, but the primary and lateral stems twine about the host plant.

Taxonomic separation of species of *Cuscuta* is based on flower and fruit characteristics (Fernald, 1950; Hitchcock *et al.*, 1959). These characteristics are often hard to discern (Austin, 1979). In his classic work on taxonomy of *Cuscuta*, Yuncker (1932) stated "The taxonomic characters are, in the main, limited to the flowers and inflorescence. The stems rarely show anything of taxonomic value".

Three species of *Cuscuta* cause economic problems in the Pacific Northwest of the USA. These are *C. campestris* Yunck., *C. indecora* Choisy, and *C. planiflora* Tenore. Contrary to Yuncker's statement, there is a difference in stem morphology among these three species. This vegetative difference is completely consistent, and can serve to separate the three species into two distinct groups without reference to floral characteristics.

The purpose of this report is to describe the difference in stem morphology in these three species, with the hope that persons interested in taxonomy of *Cuscuta* might examine additional species to determine whether this vegetative difference might serve as a critical characteristic in a key to species of the genus.

The vegetative morphology is the same for *C. campestris* and *C. indecora* (Fig. 1). At each node of the stem there is a scale leaf and a tendril. The tendril is borne outside the axil of the scale leaf and twines freely about any elongated objects it contacts. By twining about leaves and foliage of suitable host plants, the tendrils maintain connection for support of the parasite. The primary stem never twines, but continues to grow in a more or less straightforward manner. Lateral branches may or may not develop from buds in the axils of the scale leaves. These branches duplicate the growth habit of the primary shoot, and do not twine.

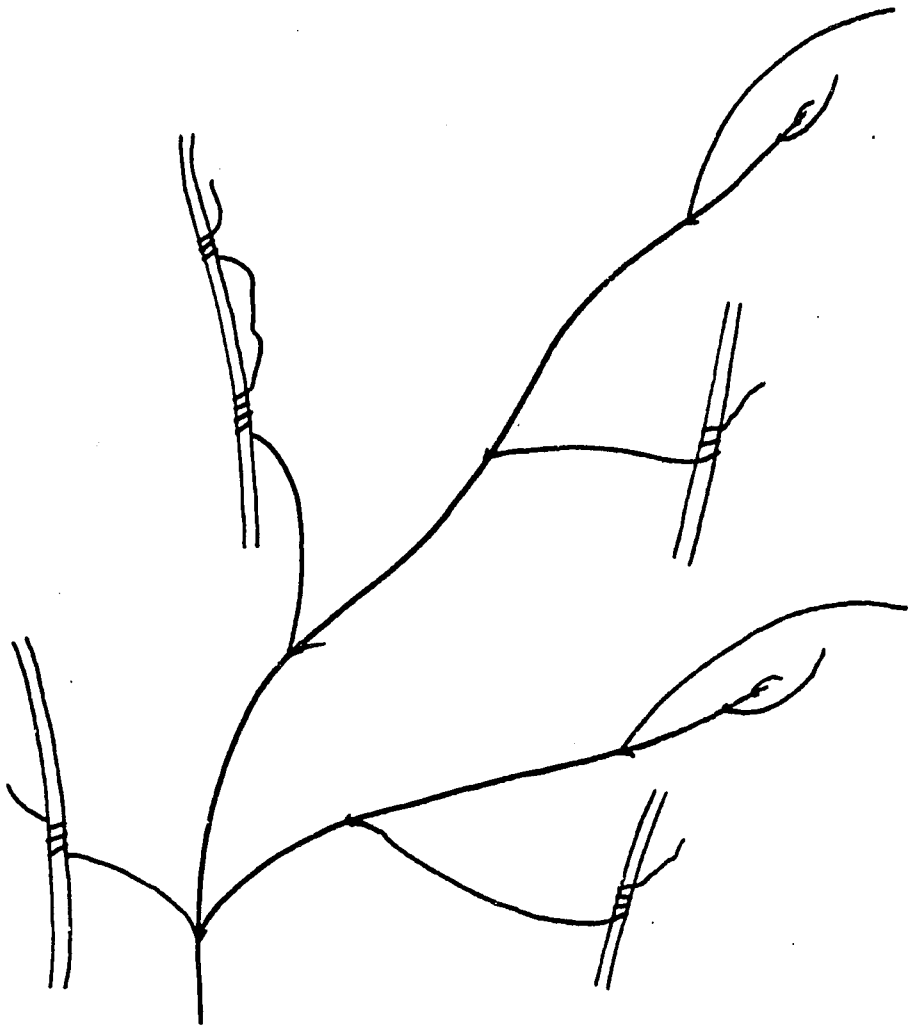

Fig. 1. Growth pattern of Cuscuta campestris and C. indecora showing primary and lateral stems, which never twine, and twining tendrils arising outside the axil of the scale leaf at each node.

C. planiflora (Fig. 2) differs from the other two species in that it does not bear tendrils. The primary and lateral stems twine and are responsible for parasitic connection to the host. One or more lateral branches develop from buds within the axils of the scale leaves at most nodes. Most of the stems of C. planiflora twine before they reach a length of 30 cm. After twining about a host plant, they may grow again without twining, and then twine again.

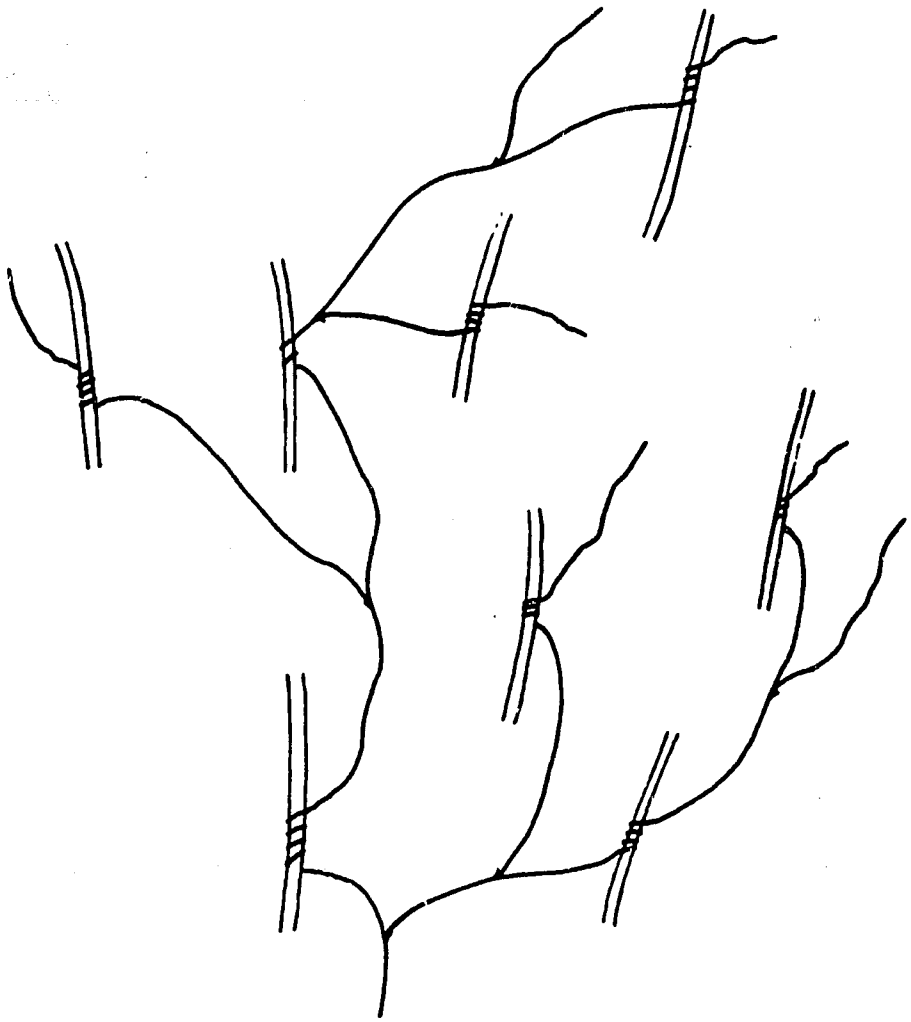

Fig. 2. Growth pattern of Cuscuta planiflora showing twining of primary stems and of lateral stems arising from axils of scale leaves, and absence of tendrils.

Tendrils of C. campestris and C. indecora not only serve the same function as the lateral branches of C. planiflora, but also resemble them. Both arise at nodes and both twine similarly. Moreover, tendrils bear scale leaves at nodes as do the lateral branches of C. planiflora. The tendrils of C. campestris and C. indecora can be positively separated from the lateral branches of C. planiflora because the tendrils arise outside the leaf axils, whereas the lateral branches arise from buds within the leaf axils (Fig. 3).

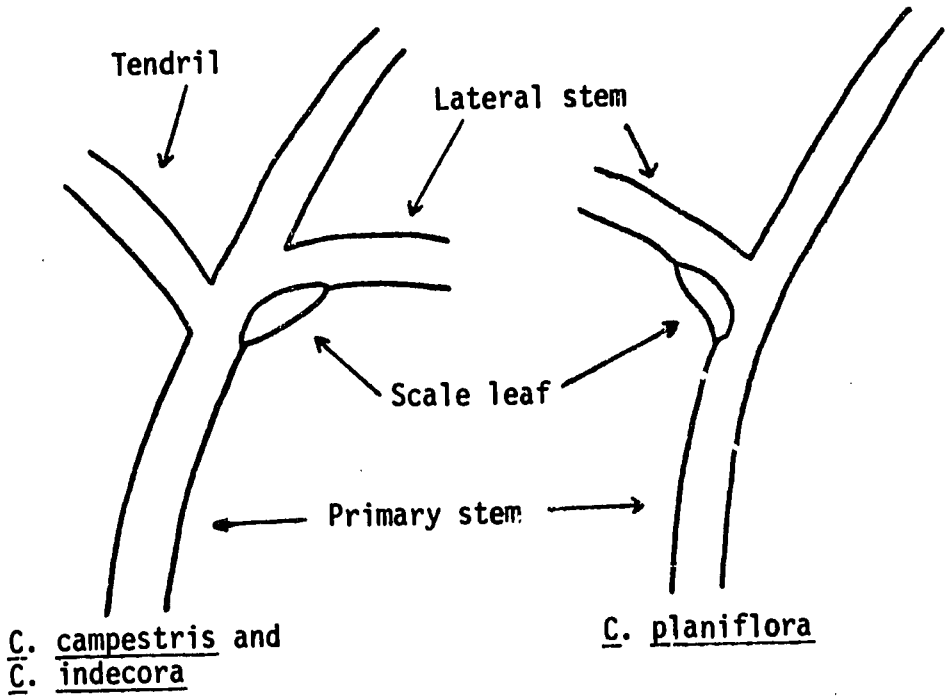

Fig. 3. Details of nodal morphology of three species of Cuscuta.

The non-twining habit of the stems of C. campestris and C. indecora allows them to grow to considerable length. Under favorable growing conditions, a single plant can form a patch several meters in diameter. In contrast, twining interrupts the forward growth of stems of C. planiflora. Individual plants therefore form very dense patches that are much smaller than those of the other two species.

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CONTROL OF CUSCUTA IN ALFALFA - A REVIEW

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Abstract. *Cuscuta* is a serious problem in alfalfa, especially that grown for seed production. Care to prevent introduction of *Cuscuta* seed onto a field and vigilance to find and destroy initial infestations are extremely important. Resistant varieties and biological control seem to have very limited possibilities. Crop rotation, tillage, and management of irrigation water are helpful in controlling *Cuscuta*. Soil-applied herbicides (chlorpropham, DCPA or chlorthal dimethyl and pronamide or propyzamide) are used to control *Cuscuta* in alfalfa, especially that grown for seed production. Certain contact herbicides (paraquat, diquat, dinoseb, and DNOC) kill attached *Cuscuta*, but are only partially selective, in that they destroy the treated alfalfa foliage, but allow subsequent regrowth of the crop from crown buds. A very low rate of glyphosate (0.075 kg/ha) applied to alfalfa foliage after *Cuscuta* has become attached controls the parasite without injuring alfalfa significantly. General weed control helps control *Cuscuta* in alfalfa by eliminating weed seedlings that serve as sites of first attachment for *Cuscuta* seedlings. Consequently, certain herbicides that do not affect *Cuscuta* directly, such as diuron and simazine, control *Cuscuta* partially by controlling broadleaf weeds.

THE PROBLEM OF CUSCUTA IN ALFALFA

The genus *Cuscuta* contains numerous species, all of which are obligate parasites that attack the stems and leaves of host species. While some species of *Cuscuta* have a narrow host range, others parasitize numerous species of dicotyledenous plants; none are known to parasitize any species of grass. Alfalfa (*Medicago sativa* L.) is a favored host of several species of *Cuscuta*, and consequently *Cuscuta* presents a very serious problem in alfalfa production.

Seeds of *Cuscuta*, which are similar in size and density to those of forage legumes, germinate independently of the presence of a host plant. Most seedlings emerge from depths of 3 cm or less (Hutchison and Ashton, 1980). The rootless seedlings emerge as arched stems (Kujawski and Truscott, 1974), which straighten and begin to rotate in a counter clockwise direction. They twine about the first elongated object they touch. If the object is the stem of a suitable host plant, haustoria penetrate the host and the vascular system of the parasite connects to that of the host (Dörr, 1972; Israel et al., 1980; Thoday, 1911; Tsivion, 1978).

The attached Cuscuta grows rapidly under favorable growing conditions and diverts the photosynthetic production of the host plant to itself (Littlefield et al., 1966). Consequently, host plants are severely suppressed, and crop production can be reduced drastically (Torell, 1967).

Alfalfa that is established as a perennial can often survive one year's parasitism by Cuscuta. In contrast, if alfalfa seeded in the spring is parasitized early in the season, the Cuscuta will usually kill the alfalfa plants.

Cuscuta seedlings emerge over a prolonged period each growing season (Dawson, 1965; Maddah, 1976), because some of the hard seeds germinate continuously as long as environmental conditions are favorable. Emergence and attachment behavior of various species of Cuscuta are similar, except that species vary in the minimum temperature required for germination (Allred and Tingey, 1964).

The seed of Cuscuta is not especially adapted for dispersal by wind or water. Most is spread by human activity. Cuscuta seed is commonly introduced into new areas as a contaminant of forage legume seed and is easily spread from contaminated areas by farm machinery.

Cuscuta in alfalfa represents three distinct problem areas. These are: a) established alfalfa grown for seed production; b) established alfalfa grown for hay production; and c) newly seeded alfalfa. Control of Cuscuta in alfalfa for seed production is further complicated, because alfalfa seed may be grown as the only crop of the season, or a crop of hay may be harvested before the seed crop is produced. In general, the problem of Cuscuta is more serious in alfalfa grown for seed production than in alfalfa grown for forage production. The frequent cutting of hay tends to suppress the growth of Cuscuta and limits (but does not eliminate) the maturation of Cuscuta seed. In contrast, the longer period of growth needed for maturation of the alfalfa seed crop favors the maturation of abundant seed of Cuscuta also. Harvesting machinery easily disseminates the mature Cuscuta seed within the field where it grew and to other fields. Furthermore, the seed of Cuscuta contaminating the harvested alfalfa seed can readily be spread to other fields.

METHODS FOR CONTROLLING CUSCUTA

Prevention and early destruction.

The best method for controlling Cuscuta in alfalfa is never to seed it. This was true before herbicides were available (Hillman, 1907; Robbins and Egginton, 1918), and remains true today. Because the introduction and spread of Cuscuta results primarily from man's activity, careful husbandry can usually prevent the introduction of Cuscuta into a field. The most important preventive measures are planting seed that is free of contamination by Cuscuta seed and by being sure that animals or machinery do not carry seed of Cuscuta into a field.

An equally important companion of prevention is vigilance. If a producer of alfalfa is constantly aware of the potential problem of Cuscuta, he can detect any patches of the parasite and destroy them before they have a chance to produce seed and spread the infestation. Scattered patches can be controlled by any method that destroys the parasite along with the alfalfa foliage to which it is attached. Cutting the alfalfa stems below the point of Cuscuta attachment, burning the foliage completely to the level of the soil surface, or applying a contact herbicide to destroy all above ground vegetation are all effective methods for destroying individual patches of Cuscuta. Possibly the most efficient method involves cutting the infected area or applying a contact herbicide followed later by complete burning of the spot after the plant material has dried (Torell, 1968). The destruction of scattered patches in a light infestation of Cuscuta is extremely important. Such treatment is inexpensive, and it will prevent the development of a general infestation, which would require expensive broadcast treatments.

Resistant varieties

Since Cuscuta is an obligate parasite, varieties of alfalfa resistant to its attack would seem a logical method of control. Some research on varietal resistance of legumes to Cuscuta has been conducted (Lilienstern, 1931), but the results have not been encouraging. Considering that several species of Cuscuta attack alfalfa, and that many of these species attack a wide range of host species, it seems unlikely that resistance to Cuscuta would be found within alfalfa.

Biological control of Cuscuta.

Several authors have investigated the effect of insects (Anderson, 1974; Baloch, 1968; Baloch et al., 1967a, 1967b, 1969; Frilli, 1966; Marikovskii and Ivannikov, 1966; Tyurebaev, 1977) and disease organisms (Leach, 1958; Oganyan, 1981; Rudakov, 1961) on Cuscuta. Although several organisms were reported to attack Cuscuta selectively, their destructive effect tended to be slow and incomplete. They therefore probably would not consistently protect alfalfa from the severe and rapid damage inflicted by Cuscuta parasitism.

Cultural control methods.

Various cultural control methods are important in controlling Cuscuta. These can form an important part of integrated management systems.

Because Cuscuta will not parasitize grasses, it can be controlled by crop rotation. Cuscuta is no problem in maize, small grains, or pasture grasses. During the production of a grass crop, all broadleaf weeds must be controlled completely, so that Cuscuta does not parasitize them and add Cuscuta seed to the soil. In the absence of a suitable host plant, seedlings of Cuscuta will die. Growing a grass crop will thus reduce the reservoir of Cuscuta seed in the soil. It will not eliminate all seeds because some will live for many years.

Although Cuscuta does not depend upon its own photosynthesis to live, light is essential for its normal development (Lane and Kasperbauer, 1965, Zimmermann, 1962), and the shade of dense alfalfa foliage will control Cuscuta almost completely (Dawson, 1966a). The shade of dense alfalfa can provide an important measure of control during the latter part of the season in alfalfa grown for seed production (Dawson et al., 1965), provided other means have controlled the parasite during the period before shade was complete.

Various tillage implements can be used in established alfalfa during the early spring to control Cuscuta seedlings before they attach to a host plant. Any tillage instrument that stirs the soil surface thoroughly without damaging the alfalfa crown can be used. Early season tillage is especially useful where alfalfa seed is grown as the only crop of the season (Dawson et al., 1965). Besides killing seedlings of Cuscuta and other weeds, such tillage can delay the growth of the alfalfa somewhat, which may be essential to control the time of blossom for maximum pollination. Besides killing Cuscuta seedlings directly, tillage also hastens drying of the soil surface. Under irrigated conditions, Cuscuta can be controlled for a period by delaying irrigation to maintain a soil surface sufficiently dry that Cuscuta cannot germinate and emerge (Dawson et al., 1965).

Alfalfa normally should not be seeded in fields known to be infested with Cuscuta (Torell, 1968). However, seeding in such soil is sometimes justified if all other conditions are favorable for alfalfa seed production. The extreme devastation of newly seeded alfalfa by parasitism from Cuscuta can be avoided by seeding the alfalfa in the late summer or fall rather than in the spring. In the Pacific Northwest of the USA, alfalfa seeded in late August or early September in soil heavily infested with Cuscuta was not injured (Dawson, 1971a). Abundant Cuscuta seedlings emerged and became attached to the alfalfa seedlings. They did not harm the alfalfa because the ensuing cool weather of autumn inhibited the development of the Cuscuta while the alfalfa seedlings continued to grow into the late fall. Freezing weather eventually killed the topgrowth of the alfalfa, together with all of the attached Cuscuta. When the alfalfa resumed growth the following spring, it was large enough to tolerate treatments for control of Cuscuta in established alfalfa.

Soil-applied herbicides for control of Cuscuta.

Soil-applied herbicides can control Cuscuta in established alfalfa during the period when cultural control methods are not effective. Such herbicides kill Cuscuta seedlings before they become attached to the alfalfa. Isopropyl m-chlorocarbanilate (chlorpropham, previously called CIPC) was the first soil-applied herbicide to be used for control of Cuscuta (Lee and Timmons, 1955; 1956), and has become a standard herbicide used for controlling Cuscuta in alfalfa (Dawson et al., 1965; Torell, 1973). Chlorpropham applied evenly to moist soil before Cuscuta becomes attached to the host plant controls Cuscuta very dependably (Dawson, 1966b). Unfortunately, control from the standard rate of 6.7 kg/ha lasts for only about one month (Dawson, 1966b; Dawson, 1969), whereas control is

needed during about four months each year. Of several other herbicides found to control Cuscuta (Dawson, 1967), most also are of short soil residual activity (Dawson, 1969).

A major part of my research on control of Cuscuta has involved a quest for herbicides that control Cuscuta for longer periods than chlorpropham does, and for methods to prolong the period of control from chlorpropham.

The short persistence of chlorpropham in soil results from loss as a vapor into the atmosphere (Slater et al., 1969; Dawson 1979), and from microbial decomposition in the soil (Kaufman and Kearney, 1965). Certain methylcarbamate compounds inhibit the microbial decomposition of chlorpropham (Kaufman et al., 1970). Applying p-chlorophenyl N-methylcarbamate (PCMC) with chlorpropham significantly prolonged the period of Cuscuta control from the herbicide in the greenhouse (Dawson, 1969) and in the field (Dawson, 1972).

Although the addition of PCMC significantly improved the control of Cuscuta with chlorpropham, PCMC has not become commercially available for this use. The commercial insecticide 1-napthyl N-methylcarbamate (carbaryl), which is chemically related to PCMC, has been shown to inhibit the breakdown of chlorpropham (Kaufman et al., 1970). It is registered with the U.S. Environmental Protection Agency for use on alfalfa. In field and greenhouse experiments, carbaryl prolonged the period of Cuscuta control from chlorpropham, but was less effective in this respect than was PCMC (Dawson, 1984). Alfalfa seed growers in the Pacific Northwest of the USA applied a tank mixture of chlorpropham plus carbaryl for Cuscuta control in 1982 and 1983. In general, control of Cuscuta was improved enough to make the addition of carbaryl to chlorpropham worthwhile.

In addition to chlorpropham, several other esters of m-chlorocarbamic acid controlled Cuscuta effectively (Dawson, 1967), but most were also short-lived in soil (Dawson, 1969). Of these herbicides, D(-) 3-chlorophenylcarbamoyloxy)-2 N-isopropylpropionamide (RP-11755) at 3.3 kg/ha controlled Cuscuta three times as long as did chlorpropham in the greenhouse (Dawson, 1969). In the field, RP-11755 at 3.3 kg/ha consistently controlled Cuscuta as well as or better than did chlorpropham at 6.7 kg/ha (Dawson 1971b). No other uses for RP-11755 were developed, and the control of Cuscuta in alfalfa did not represent a great enough potential market to justify the commercial development of the herbicide. Consequently, farmers have never had access to the improved Cuscuta control from this herbicide.

Limited greenhouse work demonstrated that the period of Cuscuta control from chlorpropham can also be prolonged by formulating the herbicide by microencapsulation as a method of controlled release (Dawson, 1976).

Dimethyl tetrachloroterephthalate (DCPA or chlorthal dimethyl) at 11.2 kg/ha controlled Cuscuta completely for 11 weeks (compared to 3 weeks for chlorpropham) in the greenhouse (Dawson, 1969). In the field, DCPA controlled Cuscuta in established alfalfa (Bayer et al., 1965; Billot et al., 1969; McNeely et al., 1966) and was also selective in newly seeded

alfalfa (Billot et al., 1969; Dawson, 1971a). It is recommended for control of Cuscuta in established alfalfa (Dawson et al., 1965; Torell, 1967; Torell, 1968). DCPA and chlorpropham are used about equally for controlling Cuscuta in alfalfa in the USA.

Another herbicide that controlled Cuscuta effectively is 2,6-dichlorobenzonitrile (dichlobenil). In both field and greenhouse studies, the period of control of Cuscuta from dichlobenil at 2.2 kg/ha was similar to that from chlorpropham at 6.7 kg/ha (Dawson, 1969, 1970). In addition to controlling Cuscuta, dichlobenil controls nearly all weed seedlings and certain established perennial weeds as well. However, it has not been used widely to control Cuscuta in alfalfa because it is costly.

Rather high rates (13.4 kg/ha) of 2-sec-butyl-4,6-dinitrophenol (dinoseb) sometimes controlled Cuscuta as well as did chlorpropham at 6.7 kg/ha, but was never superior to this standard treatment. Consequently, soil applications of dinoseb are not used to control Cuscuta in alfalfa.

The soil-applied herbicide that has become available for control of Cuscuta in alfalfa most recently is 3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide (pronamide or propyzamide). Pronamide at 1.7 kg/ha controlled Cuscuta completely in the greenhouse for periods of 6 to 16 weeks (compared to 4 weeks or less for chlorpropham at 6.7 kg/h) and in the field for about 1 month longer than did chlorpropham at 6.7 kg/ha (Dawson, 1978). Pronamide is now used in the USA to a limited extent to control Cuscuta in fields of alfalfa grown for seed production.

The herbicide (±)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate (ethofumesate) at 2.2 kg/ha controlled Cuscuta in the greenhouse for 10 weeks compared to 3 weeks for chlorpropham (Dawson, unpublished data). It also controlled Cuscuta in the field (Giannopolitis, 1979), but injured alfalfa severely (Dawson, unpublished data).

Combinations of soil-applied herbicides have controlled Cuscuta very effectively in alfalfa (Dawson, unpublished data). A full rate of DCPA (11.2 kg/ha) combined with a half rate of chlorpropham (3.7 kg/ha) or dichlobenil (1.1 kg/ha) controlled Cuscuta very well. The highly active chlorpropham or dichlobenil controlled early emerging Cuscuta very effectively at a time when DCPA was sometimes only partially effective, and the DCPA seemed to persist long enough to control Cuscuta for a period after the chlorpropham or dichlobenil had dissipated. Although expensive, a three-way combination of DCPA at 11.2 kg/ha, chlorpropham at 3.3 kg/ha, and dichlobenil at 1.1 kg/ha controlled Cuscuta completely and with favorable longevity, and controlled nearly all other weeds as well. Established alfalfa tolerated this treatment satisfactorily.

Herbicides applied post-attachment.

Certain inorganic chemicals such as lime sulfate and sulfuric acid were suggested many years ago as spot treatments to destroy attached Cuscuta and the host foliage to which it was attached (Dewey, 1898). Hillman (1907) reported that copper sulfate and iron sulfate were not satisfactory.

Several authors have reported the application of modern contact herbicides such as 1,1-dimethyl-4,4-bipyridinium ion (paraquat), 6,7-dihydrodipyrido[1,2-α:2',1'-c]pyrazinediium ion (diquat), 2-sec-butyl-4,6-dinitrophenol (dinoseb), dinoseb acetate, and 4,6-dinitro-o-cresol (DNOC), and solutions of inorganic salts to alfalfa infested with Cuscuta (Gimesi, 1966, 1979a; Halalau and Sharp, 1970; Sarpe et al., 1973; Spasic, 1967; Samoladas, 1968). These treatments do not remove attached Cuscuta selectively, but kill both the Cuscuta and the alfalfa foliage. They can be considered partially selective, because the alfalfa regrows from crown buds after treatment.

Recent work has shown that O-ethyl-O-(3-methyl-6-nitrophenyl)-N-sec butyl phosphorothioamidate (H-26905), DCPA, and N-(phosphonomethyl)-glycine (glyphosate) controlled Cuscuta selectively in alfalfa when applied to the alfalfa foliage and the Cuscuta after Cuscuta was attached to the alfalfa (Dawson and Saghir, 1983). DCPA was effective at the rather high rate of 10 kg/ha and H-26905 is not presently being developed for commercial use. Of the three herbicides, glyphosate is of greatest interest because it controlled Cuscuta at the very low rate of 0.075 kg/ha.

Glyphosate affected the Cuscuta shoots that were present at the time of treatment about equally whether applied direct to the Cuscuta itself or translocated from the treated alfalfa foliage. Glyphosate applied to a Cuscuta shoot killed the treated tissue, but did not affect imbedded haustoria or any portion of the parasite basipital to the portion treated. Cuscuta can regenerate freely from haustoria within the host stem, even if all visible portions of the parasite are removed (Truscott, 1958). Therefore, glyphosate applied to alfalfa foliage is especially effective, because it contacts the imbedded haustoria during translocation to the stems of the parasite.

Field research in 1983 confirmed the selective control of Cuscuta on alfalfa from post-attachment applications of glyphosate (Dawson, unpublished data). The margin of crop tolerance was narrow, but adequate. A dosage of 0.075 kg/ha stopped the growth of Cuscuta and suppressed its regrowth with little, if any, noticeable effect on alfalfa. A dosage of 0.15 kg/ha controlled Cuscuta more completely, but usually arrested alfalfa growth somewhat. Suppression of established alfalfa increased as the rate of glyphosate increased up to 0.3 kg/ha, the highest rate applied. The effects of all rates of glyphosate on established alfalfa were temporary, and the plants recovered fully. Alfalfa was more susceptible to injury from glyphosate as seedlings than as established plants. A dosage of 0.3 kg/ha applied to seedlings that had only three trifoliolate leaves killed about one-third of the plants. Surviving plants were retarded, but

recovered fully and then grew normally. Lower rates of glyphosate did not kill any plants at the three trifoliolate leaf stage. The highest rate applied, 0.3 kg/ha, did not kill any plants with eight or more trifoliolate leaves.

Glyphosate controlled Cuscuta more effectively when applied to established alfalfa that was more than 30 cm high than when applied to alfalfa with less foliage or to newly seeded alfalfa. Control was most effective when applied before the Cuscuta had budded, but after its stems had reached a length of 30 cm. Shade from the alfalfa foliage evidently helped to retard the recovery of the Cuscuta. Glyphosate appeared to injure alfalfa less when it was infested with Cuscuta than when it was free of Cuscuta. Most likely this is because much of the herbicide moved from the alfalfa to the parasite, rather than to the growing points of the alfalfa itself.

In the 1983 research, no application of glyphosate killed all of the attached Cuscuta. Some of the haustoria within some of the alfalfa stems always survived and new shoots regenerated from this surviving tissue. Glyphosate applied before the Cuscuta had injured the alfalfa noticeably saved the alfalfa from severe damage. Thus, the use of low rates of glyphosate appears very promising for protecting alfalfa grown for forage production from Cuscuta injury. Since control of Cuscuta must be complete to be satisfactory in alfalfa grown for seed production, the use of glyphosate appears to be less promising in alfalfa grown for seed production than in alfalfa grown for forage production.

A herbicide related to glyphosate, trimethylsulfonium carboxymethylaminomethylphosphonate (SC-0224), also controlled Cuscuta selectively in alfalfa in the 1983 field experiments. At all rates, its effects on alfalfa and Cuscuta were similar to those of glyphosate.

Benefit from general weed control.

Control of annual weeds in general can assist in control of Cuscuta in alfalfa. In a weedy field, much of the Cuscuta that infests alfalfa first becomes attached to seedlings of broadleaf weeds. Any program that controls these weeds reduces the possibility that emerging Cuscuta seedlings will attach to a host plant. This is especially true in alfalfa grown for seed production, where the alfalfa plants are usually more widely spaced than in fields of alfalfa grown for forage. If weeds are controlled, a high percentage of emerging Cuscuta seedlings die because they cannot reach a host plant. For this reason, certain herbicides that control other weeds selectively in alfalfa, will assist in Cuscuta control, even though they do not control Cuscuta directly. Examples of such herbicides are 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), S-ethyl dipropylthiocarbamate (EPTC), 4-amino-6-tert-butyl-3-(methylthio)-as triazin-5(4H)one (metribuzin), 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine), and 3-tert-butyl-5-chloro-6-methyluracil (terbacil) (Dawson, 1967). A combination of N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine (pendimethalin), 3-(3,4-dichlorophenyl)-1-methoxy-1-

methylurea (linuron), and diuron applied to dormant alfalfa controlled numerous weed species, including Cuscuta (Gimesi, 1979b). This is a mixture of a herbicide that kills Cuscuta (pendimethalin) and two that do not (diuron and linuron).

Integrated control systems.

A single control method by itself seldom controls Cuscuta in alfalfa for the entire season. In the Pacific Northwest of the USA, integration of several control methods protects alfalfa grown for seed production for the necessary four months (Dawson, et al., 1965; Dawson, et al., 1984). A typical schedule begins with broadcast tillage from mid March to mid April. Chlorpropham at 6.7 kg/ha is applied in mid April and reapplied in mid May. Irrigation water is not applied in early and mid June, so the soil surface becomes dry and no Cuscuta seeds germinate. By mid June, the cover of alfalfa foliage is dense enough to suppress any Cuscuta seedlings that do emerge. Any Cuscuta that emerges after mid July does not have time to harm the alfalfa, even under favorable growing conditions, before chemical defoliants are applied in late August or early September in preparation for seed harvest.

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CYTO-PHYSIOLOGICAL ASPECTS OF *OROBANCHE CRENATA* FORSK. PARASITIZING *VICIA FABA* L.

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Abstract. The present study describes *Orobanche crenata* Forsk. parasitizing *Vicia faba* L.

In response to a host root stimulus, the seeds of the *Orobanche* germinate and give rise to a delicate organ, the "procaulôme". Both the nature and function of this organ are discussed on the basis of the new histological data.

After a rapid attachment to the host root, a part of the seedling, the endophyte, penetrates into the host tissues. The part remaining outside gives rise to a tubercle which develops one single shoot apex and many short roots.

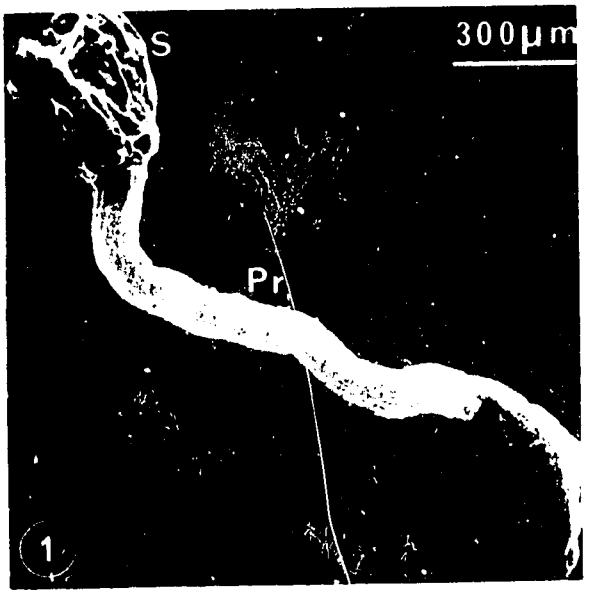
The endophyte never crushes host cells during its penetration. As soon as the first cells reach the host vessels, they differentiate into xylem elements, establishing a direct connection. Such a connection has never been observed between the phloems of the two plants. Our physiological data suggest that sucrose may be the main metabolite involved in the transfer of organic substances from the host to the parasite.

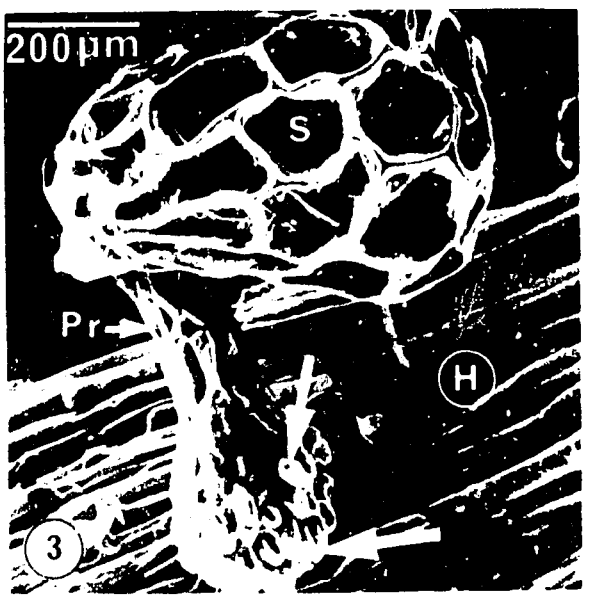
INTRODUCTION

Orobanche spp. are common pests in the Middle East, around the Mediterranean and in Europe. These parasitic angiosperms cause damage in the crops attacked. In some cases, the diminution of yields is so drastic that cultivation must be given up (Kasasian, 1971 ; Lubenov, 1973 ; Aber, 1983).

By now, the biological cycle of *Orobanche* spp. is well known. The minute seeds of *Orobanche* germinate only in the vicinity of certain plant roots. They give rise to an organ called "procaulôme" by Velenovsky (1905). It has also been named radicle (Chabrolin, 1939 ; Roger, 1954), germ-tube (Krenner, 1958) or germ-tube-like organ (Abou-Raya *et al.*, 1973). After attachment to a host root, *Orobanche* develops an absorptive system or haustorium (Kuijt, 1977 ; Ozenda and Capdepon, 1979) which penetrates through the cortex and then goes to the vascular cylinder. There, more or less direct connections are established between the conducting elements (xylem and phloem) of the two plants (Toth and Kuijt, 1976 ; Musselman, 1980). These connections are very important because they represent the channel through which nutritive substances can move from the host to the parasite. The outer part of the seedling develops into a tubercle which gives rise to a spike.

However, some aspects of the biology of these plants remain unclear : 1- the ontogeny and the ultrastructure of the "procaulôme", 2- the fine morphogenesis of the seedling and particularly the tubercle and the haustorium,





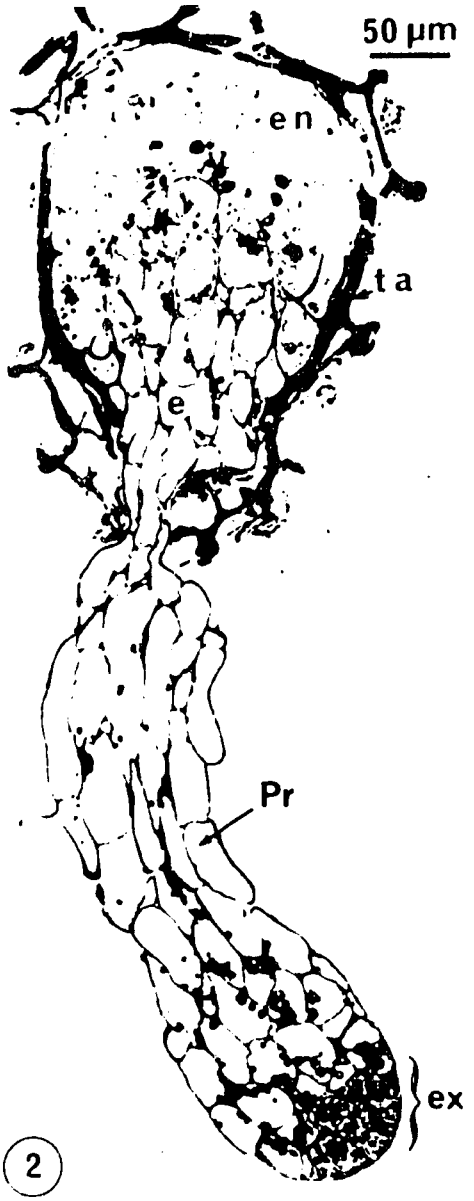


Fig. 1 - SEM. Aspect of a germination with its long "procaulôme", Pr. S, seed.

Fig. 2 - Longitudinal section of a germination. ex, extremity of the "procaulôme", Pr ; e, rests of the embryo ; en, endosperm ; ta, testa. Semi-thin section ; PAS-Regaud's hematoxylin.

Fig. 3 - SEM. Very beginning of the fixation on the host, H. The tip of the "procaulôme", Pr, differentiates papillae, arrows. S, seed.

3- the nature and the importance of the sugar uptake in the host, according to the main phenological stages established previously (Aber and Sallé, 1983 a).

These unanswered questions and the damage caused by *O. crenata* Forsk. in Morocco, lead us to study the morphogenesis and the sugar nutrition of *O. crenata* parasitizing broad bean (*Vicia faba* L.).

METHODS AND MATERIALS

Mature seeds of *O. crenata* and of broad bean (*Vicia faba* major var. 269) were collected in Morocco in 1979. They were grown together in pots according to the conditions described by Aber (1981). For light and transmission electron microscopic study, seeds, "procaulômes" and seedlings were treated as described earlier (Aber, 1981). For scanning electron microscopy (SEM) study, samples were fixed with 4% glutaraldehyde and 1% OsO_4 and then treated by the Cohen and Garner's method (1971).

In order to determine the nature and the importance of sugars taken up from the host, parasitized plants of broad beans were exposed to $^{14}CO_2$ in a container, under illumination. For each seedling of *O. crenata*, the radioactivity was visualized by autoradiograms and estimated with a scintillation counter. Labelled substances present in the host roots and in the parasite were identified by paper chromatography (Aber *et al.*, 1983).

RESULTS

The autotrophic period of life

During germination, the seed of *O. crenata* gave rise to a delicate filamentous organ, the "procaulôme" (Fig. 1, Pr). The morphological, cytological and ultrastructural work of Aber and Sallé (1983 b) showed its originality. It consisted of two parts (Fig. 2). The main core, Pr, was essentially parenchymatous whereas the extremity, ex, was composed of about ten isodiametric cells. The latter were active with dense cytoplasm, voluminous nucleus, reduced vacuome and poorly differentiated plastids and mitochondria. The cap and the typical meristem of roots were missing. Moreover, it is important to notice that the "procaulôme" was devoid of any conducting elements.

With the numerous germinations obtained in our laboratory, we followed the morphological evolution of the "procaulôme". During the early germination, the "procaulômes", using the material stored in the endosperm, developed straight. Then, most of them grew towards the root which had provoked their germination. Moreover, we observed that only secondary roots were parasitized whereas the main one remained unattacked, presumably due to their structural features.

As soon as the tip of the "procaulôme" contacted a suitable host root, it flattened and differentiated papillae (Fig. 3, arrows).

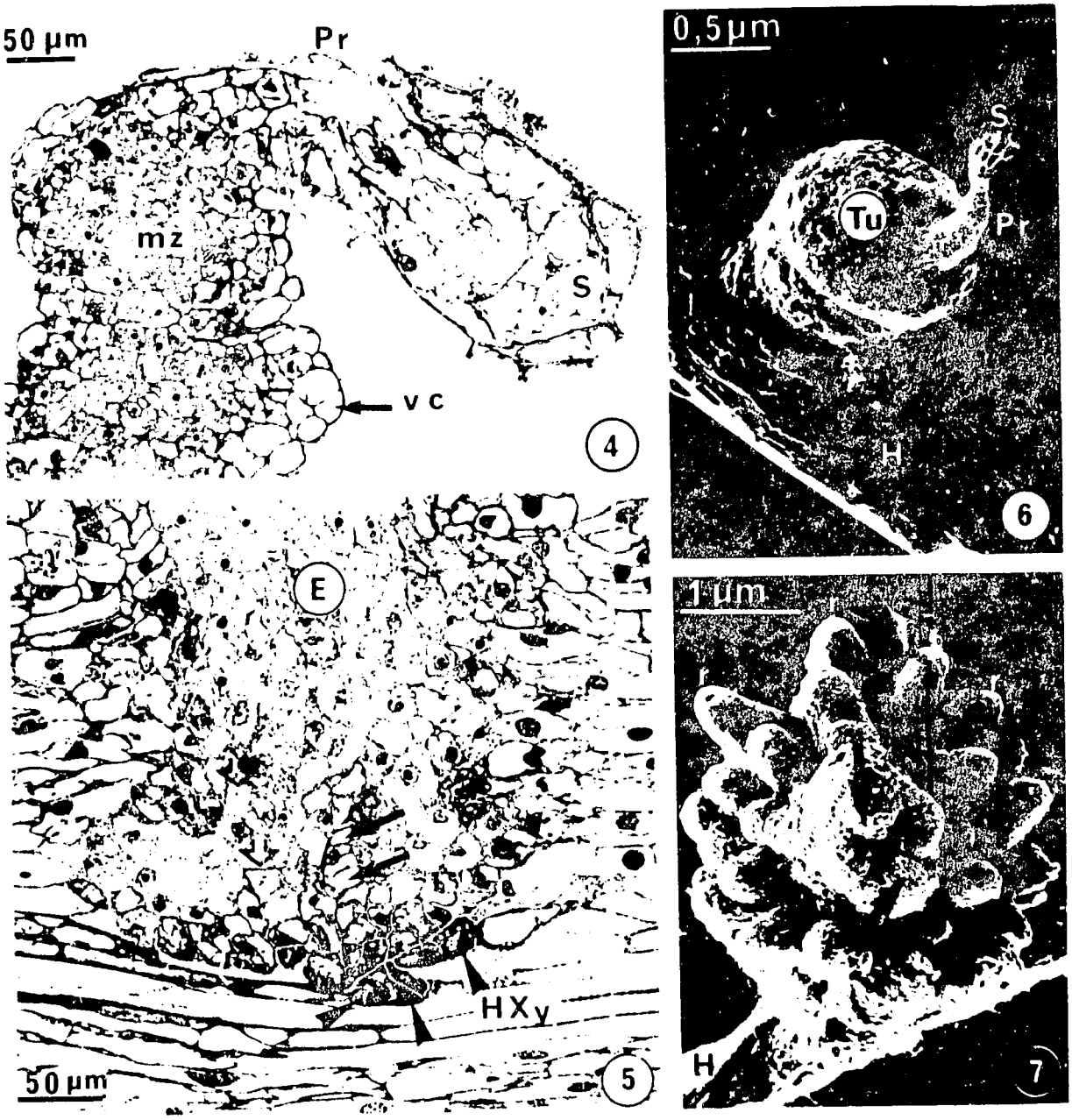


Fig. 4 - Detail of the outer part of the seedling. The meristematic zone, mz, is covered with vacuolated cells, vc. Pr, "procaulôme" ; S, seed.

Fig. 5 - Detail of the inner part of the seedling or endophyte Ⓔ . ⟶, sieve cell ; ⇨, differentiating xylem elements ; ➤, apical cells with a dense cytoplasm. HXy, host xylem. Note the absence of cell remnants at the limit between the two plants.

Fig. 6 - SEM. Young tubercle, Tu, with "procaulôme", Pr, and seed, S, still present. H, host.

Fig. 7 - SEM. Older tubercle with many bumps, r, corresponding to the future roots. Arrows, scales protecting the shoot apex.

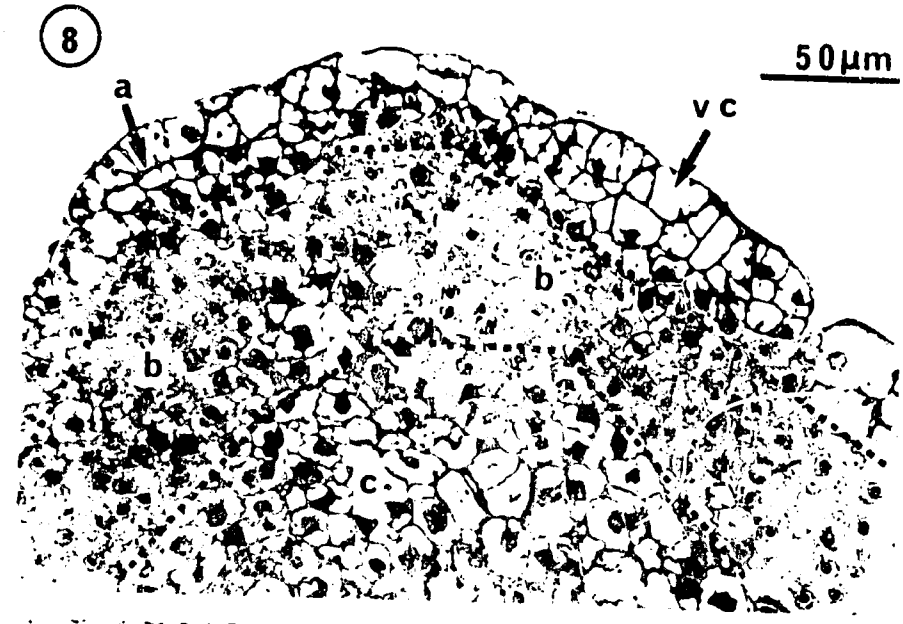


Fig. 8 - Detail of the a, b, and c zones of the tubercle. In the b region, many mitosis can be observed, arrows. vc, vacuolated cells.

Fig. 9 - Detail of the endophyte, Ⓔ. Some of its front cells differentiate into xylem elements, ➡. HXy, host xylem.

The parasitic period of life

Rapidly, the zone of contact and of penetration of the parasite swelled and became yellowish. At this stage, the seed and the procaulôme were still present. From now on, a part of the seedling will remain at the outside of the root whereas the other will form an internal endophyte. The ontogeny of these two regions was followed on semi-thin sections as presented by figures 4 and 5.

The outer part of the seedling is composed of meristematic cells covered with one or two layers of vacuolated cells (Fig. 4, vc). This meristematic zone will play an important organogenetic role because it will give rise to the tubercle. The inner part (Fig. 5, Ⓔ), which corresponded to the endophyte, passed through the epidermis and cortex and rapidly reached the vascular cylinder of the host root. Then, the tubercle developed (Fig. 6, Tu) to a size depending on the climatic conditions and on the vigour of the host. The more vigorous the host, the larger the tubercle. Its diameter could exceed 20 mm. This correlation emphasizes the importance of the nutrients available in the host and tapped by the parasite.

12 or 15 days after attachment, the tubercle surface presented many protuberances which became more and more prominent (Fig. 7, r). They were the future roots. However, in *O. crenata*, they never elongated to more than 30 mm. The shoot apex, protected by small scales (Fig. 7, arrows), will give rise to a bud and then to a spike which could be 50 cm long.

The cytological observation of young tubercles showed five different zones (Fig. 8). The outer zone (a) consisted of one or two layers of protecting vacuolated cells. Beneath them, one could find clusters of small meristematic cells (b) which divided frequently. Their morphogenetic role is obvious because they will initiate the shoot apex and numerous short roots. In the basal direction of the tubercle, the cells vacuolated rapidly and became parenchymatous (c). However, in the axis of the tubercle, they remained dense and often differentiated xylem or phloem elements. Both sides of the tubercle were occupied by parenchymatous cells involved in a storage process. The reaction with periodic acid-Schiff (PAS) showed numerous starch granules.

During the early penetration into the host root, the young haustoria were mainly meristematic. Their cells divided actively and then elongated. These two processes would develop a mechanical force which could explain the penetration of the haustorium into the host. However, as shown by figure 9 the limit between the two plants remained very clear : no cells remnants were observed. Moreover, in this region, the density of the cells indicated a high metabolic activity. These data suggested an enzymatic penetration. This hypothesis was confirmed by the rapidity of the penetration itself.

A successful implantation of *Orobanche* needed the establishment of more or less direct connections between the conducting elements of the two plants. As soon as the tip of the endophyte made contact with host xylem tissues, a gradient of differentiation was induced. Some front cells differentiated into tracheids establishing a direct connection with the host xylem (Fig. 9,

; Fig. 10). Then, the xylem differentiation progressed towards the xylem elements of the tubercle.

The situation was not so clear for the phloem. Sieve elements were observed in the tubercle and in the haustorium. However, no direct contact has ever been observed between the two phloic systems. Moreover, a thickening of the cell walls of the parasites in contact with host cells (Fig. 10, *) was frequently observed.

These ultrastructural observations clearly indicated that, if the apoplastic pathway was mainly involved in the transport of water and mineral salts, *via* the xylem, it could participate to some extend to the transfer of the photosynthates *via* the cell walls (Fig. 9, 10, *).

Transfer of organic substances from the host to the parasite.

After a short exposure of the host foliage to $^{14}CO_2$, the amount of labelled organic substances was higher in the parasite than in the host root system : $28.2.10^6$ dpm for all the parasites together and $4.4.10^6$ dpm for the host root system. On autoradiograms (Fig. 11) the parasite had always much

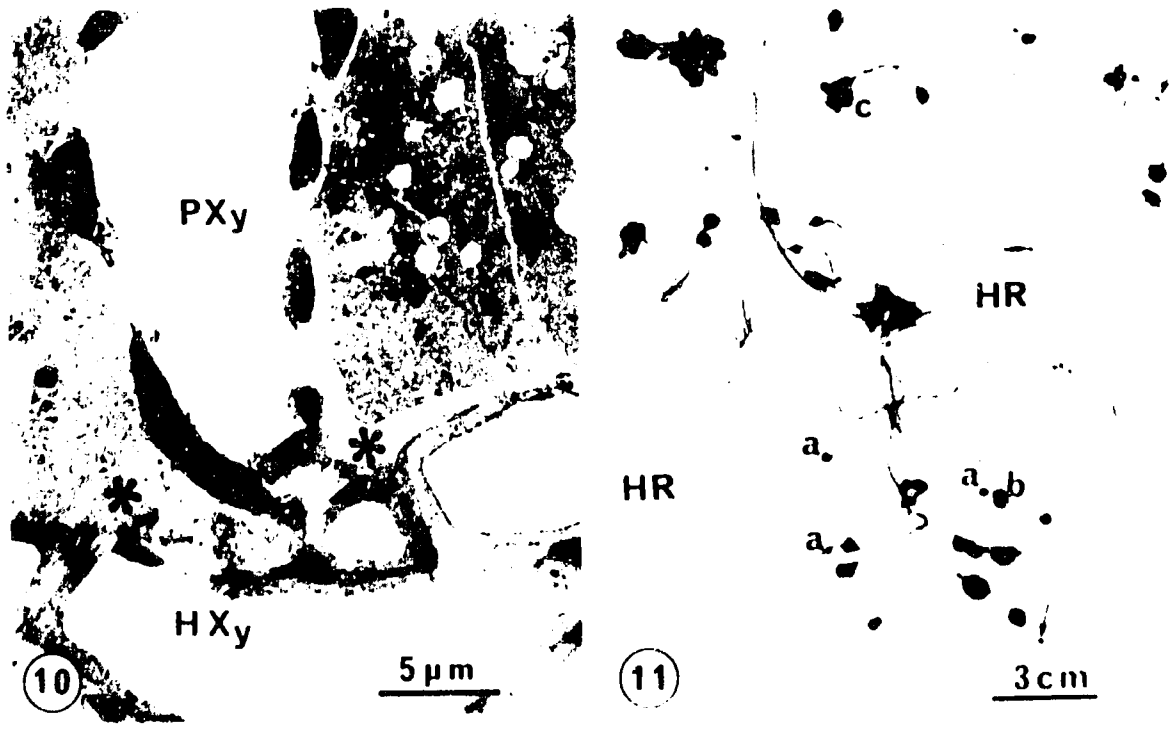


Fig. 10 - Direct connection between the host xylem elements, HXy, and the parasitic one, PXy. Notice the thickening of the host parenchymatous cell walls. *.

Fig. 11 - Autoradiograms of a host root system bearing many parasites. After a 6h exposure of the broad bean foliage to $^{14}CO_2$, tubercles of the parasite are much more labelled than the host root system. a, b and c indicate the different stages of development of the tubercles. The youngest tubercles, arrows, are easily observed. HR, host root system.

more labelling than the host, whatever its age or its development. Even the youngest tubercles, not visible without a binocular, were so labelled that they could be easily observed on the autoradiograms (thin arrows). It is important to notice that these small tubercles did not establish conducting connections with the host (Aber and Sallé, 1982).

The analysis of the labelled substances showed that about 60% of the radioactivity was contained in the neutral fraction (carbohydrates) in both host and parasite. Sucrose, glucose and fructose were identified by paper chromatography. A quantitative study proved that sucrose was the most important carbohydrate (40% of the total sugar radioactivity) present in the host and in *O. crenata*. On the contrary, glucose and fructose levels were very low (respectively 6 and 9%). This result suggested that sucrose may be the main metabolite involved in the transfer of organic substances from the host to the parasite.

DISCUSSION

This study covered the main stages of development of *O. crenata*, i.e. the germination, the fixation and the penetration.

Embedding in epoxy resins allowed a fine structural study of the "procaulôme" developed during the germination of the seed. It consists of a weak parenchymatous core tipped with a group of very active cells showing a dense cytoplasm. It is devoid of conducting elements.

Our morphological, histological and ultrastructural observations on the organ otherwise referred to as a radicle, germ-tube etc., lead us to prefer the term "procaulôme" in keeping with the originality of the organ. The "procaulôme" shows a positive tropism in regard to the root which provokes the germination of the seed (Cézard, 1973). All aspects emphasize the high level of specialization of this organ : it brings the organogenetic cells in contact with the suitable host but its feeding role seems to be very limited.

O. crenata was characterized by a rapid attachment during which papillae were differentiated. The parasite never built a massive organ of attachment as has been reported for other flowering parasitic plants : *Viscum* (Thoday, 1951 ; Sallé, 1975), *Loranthus* (Menzies, 1954), *Phthirusa* (Dobbins and Kuijt, 1974), *Comandra* (Toth and Kuijt, 1976).

This very short phenological stage was followed by a rapid penetration which certainly involves an enzymatic action. The haustorium grows directly to the vascular cylinder. This urgent need of nutrients can be explained by the little stored material present in the endosperm. However, enzymes involved in this phenomenon must be identified and their still questionable activity must be visualized by cytochemical or immunological methods.

By now, modalities of development of tubercle are quite well known. We demonstrated that the massive meristematic zone observed in early stages of penetration divides into small groups of cells which give rise to a shoot

and many root apices. This peculiar organogenesis is presumably under the control of a hormonal balance which requires further study.

Our ultrastructural observations of the conducting elements of the haustorium is not completely satisfactory because, even though direct connections between the xylem of the two plants has been demonstrated, the phloemic relations have not been completely elucidated. We never observed the peculiar phloic conduits described by Dörr and Kollman (1974, 1975) in secondary haustoria of *O. ramosa*. One can wonder if they exist in *O. crenata*. More study is required.

However, our ultrastructural observations done at the limit of the two plants demonstrated that, even if direct phloem connections do not exist, a thickening of the cell wall of the parasite which was in contact with host cells, increases the apoplastic compartment . This structural work was completed by a physiological approach of the nutrition we performed with $^{14}CO_2$. Beside the fact that transfer of carbohydrates was important from the host to the parasite (Whitney, 1972), we established that the transfer occurs in the very young tubercle even when they were still devoid of direct connections with the conducting cells of the host (Aber and Sallé, 1982). These complementary researches clearly suggest the importance of apoplastic transport. A cytophysiological study with the electron microscope is being carried out in order to identify the cells or the cellular compartments involved in such a transfer.

Already, our better knowledge of the relationships between *O. crenata* and *V. faba* should help to determine the best chemical control to use.

Acknowledgements. This work was partly supported by the C.N.R.S. (E.R.A. n° 616). Research support from D.S.E. (Deutsche Stiftung für internationale Entwicklung) is gratefully aknowledged.

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STUDIES ON THE RESISTANCE OF VICIA FABA TO OROBANCHE CRENATA

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Abstract. Parental lines and segregating generations involving inbred Vicia faba L. showing different degrees of susceptibility to Orobanche crenata Forssk. have been tested for resistance under different intensities of infestation. Data fit well to the additive model, suggesting no dominance for the genetic control of the resistance.

Path analysis performed in inbred lines, populations and segregating generations under the some intensities of infestation show that the Orobanche attack affects seeds per plant (the final effect in our study) through pods per plant and not through the number of seeds per pod. The methodology tested has proved to be useful to analyze the "path attack" of Orobanche on Vicia faba.

INTRODUCTION

It is obvious that the relationships between Vicia faba L. and Orobanche crenata Forssk. are complex for they are formed by many partial interactions at a multiple level (biochemical, histological, etc), It is by no means obvious that the final result of the host/parasite interaction, i.e., resistance or susceptibility, has to have a genetic control which probably will be a "composite" system. That is: the genes controlling the translocation of sucrose from the host to the parasite will very probably be different from those controlling the physical attachment of the germinating parasite seed on the host root etc.

So then, to speak about the genetic system controlling the resistance to broomrape without any more precision is a very rough way of tackling the problem, but it is also necessary to clear away a path in a mountain of genetic and environmental effects. The parasite can acts on the host through a multitude of ways, and it can effect several characteristics, sparing others. The knowledge of this pattern is not only necessary to split the overall genetic system for resistance into components, each one of these being more meaningful: it is also basic for the practical breeder, which will be able to select for resistance in a more effective way.

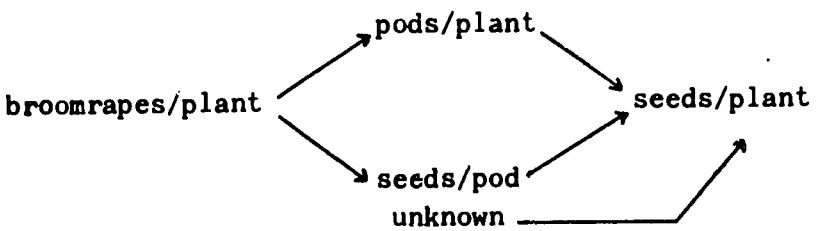
Previous studies have shown that the most likely "rough" system for resistance was no dominance or a very slight partial dominance for resistan

ce (Cubero and Moreno, 1979; Suso, 1980; Cubero and Martinez 1980; Cubero, 1983; Gil et al 1982). These results are in agreement with those obtained for Vicia sativa (Gil, et al., 1974 and this volume). We are trying now to know the different components of this general genetic system throught the study of the form Orobanche is using to attack V. faba.

In this paper we will present results of a first analysis of the relationships between the Orobanche attack on the one hand and several characteristics related to yield on the other, and also new data concerning again the overall genetic system for resistance, but following a different approach (i.e., by means of segregating generations andnot by diallel crosses). In a certain way, this study is a "training" or a "prologue" for a much more complete work to be performed in the next years. New crosses are being performed including the resistant line F 401, from Egyptian origin, previously tested under field and glasshouse conditions and after two generations of selfing at least.

METHODS AND MATERIAL

Inbred lines, populations, hybrids and segregating F_2 and F_3 generations have been studied. Paucijuga, equina and major accessions have been used for this purpose. All the these were sown in two sites of a heavily infected plot, these sites differing in the degree of infestation accoriding to previous experience (confirmed in this study). Data were recorded on individual plants in all cases. Segregating generations were studied by means of histograms, and Cavlli analysis (Mather and Jinks, 1971) was performed to test the additive model. Total and partial correlation as well as path coefficients were also obtained. Only four characteristics were recorded (parasites/host plant, pods/plant, seeds/plant and seeds/pod) according to the following causal scheme:



seeds/plant and not yield/plant was choosen as final as a way of standardizing the huge differences existing among the different accessions. This scheme will be complexed in future studies.

RESULTS AND DISCUSSION

A) Resistance vs. susceptibility

Fig. 1 shows the results of F_2, F_3 and parentals in the case of the

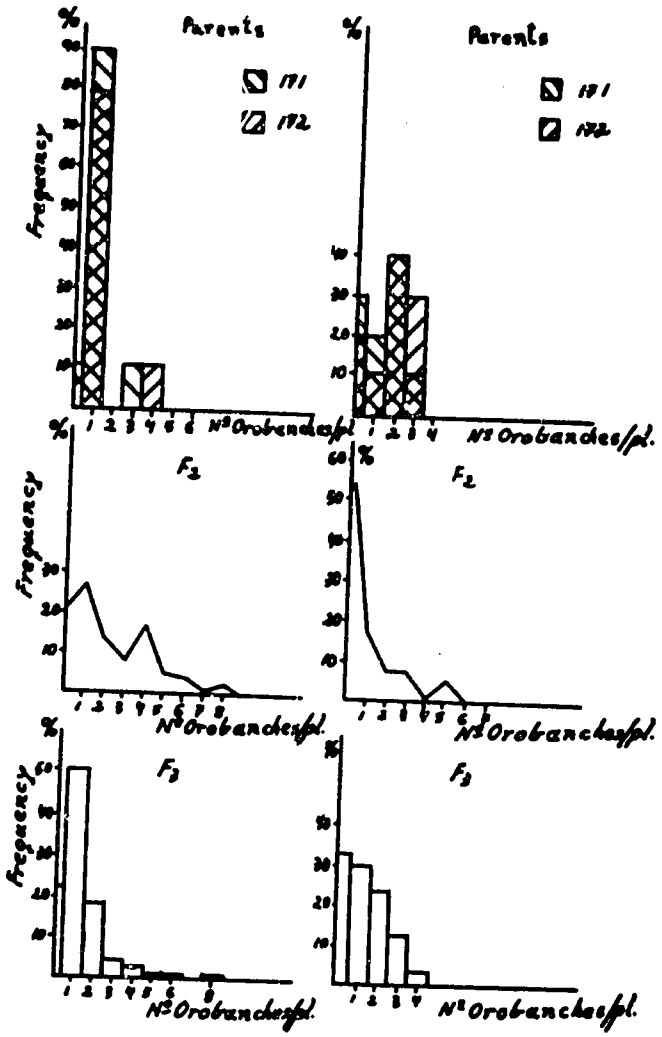


Fig. 1

Cross 172 x 171 Left: high level of infestation and right: low level of infestation.

Parentales, F_2 and F_3 of a cross between "resistant" (less susceptible) lines (171 and 172). Left: high level and right low level of infestation.

TABLE 1

Cavalli analysis of the cross 172 x 171

a) Genetic parameters. Values and significance

	infestation	
	high	low
mean	1.0 n.s.	1.7 +
additivity	0.3 n.s.	0.2 n.s.
dominance	1.4 n.s.	- 1.4 n.s.

ns: not significance +: signif. at the 5% level.

b) Test of the additive model

	High infestation		Low infestation	
Generation	observed	expected	observed	expected
P_1	1.1	1.0	1.7	1.9
P_2	1.2	0.9	1.3	1.4
F_2	1.7	1.6	0.8	0.9
F_3	1.2	1.3	1.4	1.3
	$x_1^2 = 0.13$		$x_1^2 = 0.06$	
	$P > 0.70$		$P > 0.80$	

cross 172 x 171, both lines having relatively low susceptibility among the material we have handled up to now. There are some differences between the two zones of the plot (high and low level of infestation), even when the overall picture is similar in both cases, mainly in comparison with fig. 2. The graphs of fig. 1 are not very indicative of the genetic system involved in the resistance. Both F_2 and F_3 show larger ranges of parasitic shoots per host plant than those of the parental lines, but obviously it is not enough to accept transgressive inheritance. In other words; the genes expressed in the segregating generations are present in both parentals. Mean values of the four generations are rather similar: only the F_2 in the less infected zone is less infected indicating a possible direction towards dominance for resistance, but very weak in any case. The conclusion of the line F 402 in the crosses will help in these studies.

Cavalli analysis (table 1) shows no significance of the genetic parameters, but it is worth mentioning that this test is not very sensitive in detecting genetic parameters. On the contrary, expected values fit well the observed ones. The additive model can be admitted as it would be expected in a case of total or almost total absence of dominance.

Fig. 2 shows a very different pattern. These F_2 and their parental susceptible lines, sown in the highly infected zone, clearly show the absence of resistance when compared to the results in Fig. 1. The parental lines were also sown in the zone with less infestation and the distribution was similar to that shown in fig. 2. It is the difference between figs. 1 and 2 the fact demonstrating the existence of resistance in V. faba, as it has been shown in previous papers (Cubero 1983, for example). That this resistance is not enough to be used in commercial cultivars is different matter that probably will be solved by the use of new strains as F 402. Crosses between "resistant" and susceptible lines were analyzed in previous papers with the results already mentioned above of no dominance or very slight partial dominance for resistance.

B) Correlation

As in the previous case, only a few of the results obtained are shown here to avoid unnecesary repetion of similar figures.

B1) Broomrapes/plant-pods/plant and seeds/plant.

The results are presented altogether because of their similarity, a fact due to the high correlation coefficient between pods and seed per plant, close to unity in most of the cases and always higher than 0.93.

The values obtained are highly significant (1% level) in most cases, and they are rather independent of the kind of material studied, i.e., inbred lines, populations, F_2 and F_3. The values range from -0.10 to -0.55.

When the same material has been studied under high and low levels of infestation, the change of the values observed does not show any visible

pattern. In some cases the correlation coefficient diminishes and in others increases. Even two replications of the same material in the same zone of the plot can show drastic differences in the value of the correlation coefficient (for example, from -0.28 to -050, from -0.17 to -0.46 etc, with a change from non significance to significance). The comparison between resistant and susceptible materials does not produce clear results either. Some coefficients of very susceptible lines and F_2 were high, but others were as low as those of the most resistant materials (in this case F 402, that was tested only as inbred line).

Concerning the origin of the material, the only visible results is that the highest values (over -0.50) have been shown by Spanish material but the distribution has been continuous from -0.10 to -0.73. It remains to be tested whelth the autocthonous broomrape is reacting in a more virulent way on some Spanish genotypes. But even in this case the results are of not easy interpretation: two of the most susceptible lines (VF 114 and VF 119) gave very different values for the correlation coefficients (-0.52 AND -0.22^{ns} respectively) and its F_2 from -0.73 to -0.64 in different repetitions. The F_2 between other very susceptible line (VF 26: v = -0.51) and both VF 114 and VF 119 gave -0.32 (26 x 114) and from -0.47 to -0.62 (26 x 119) (all these values were obtained from different repetitions but in the same site of the plot).

Finally, comparing inbred lines and F_2 simultaneously in both levels of infestation does not produce any clear pattern; either some times the correlation coefficient decreases when passing from the high to the low level and in some other cases the reverse is true.

If instead of total correlation coefficients we obtain the partial correlation ones (that is, we obtain the correlation coefficient between 1 and 2 removing the effects of 3 and 4), the picture changes dramatically. The correlation between parasite number and pods per plant removing the effects of seeds/pod and seeds per plant is nil in almost all cases (only 5 exceptions in more than 80 observations, and only one out of the 5 cases was highly significant). The same result was obtained for the correlation between parasite numbers and seeds per plant removing the effects of seeds per pod and pods per plant. Again it is a logical result given the high correlation existing between seeds and pods per plant (the partial correlation coefficient between these characteristics removing the effects of O. crenata per plant and seeds per pod, also was superior to 0.90).

When the partial correlation coefficient between parasites and seeds per plant is obtained, removing only the effect of seeds per pod, their values are very similar to the total correlation coefficient. This result is also a consequence of the high correlation between pods and seeds per plant.

Many causes can be mentioned to explain such results: strong environmental influence (a thing already known as acting very strongly in the Orobanche attack), interactions between host genotypes and parasite races, in-

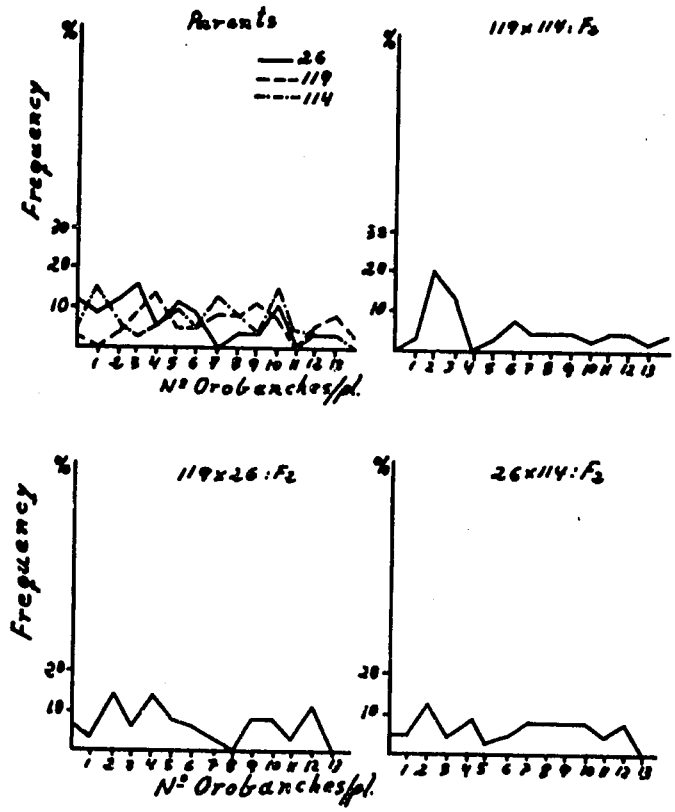


Fig. 2

F_2 of three crosses between susceptible lines. High level of infestation.

TABLE 2

Multiple regression coefficients with standardized variables

Material	Infestation	Coefficients k_1	k_2	k_3
- Inbred lines				
172 (pauc.)	High (H)	-0.30	1	0.36
	Low (L)	-0.37	0.98	0.56
26 (eq.)	H	-0.45	0.90	-0.03
1071 (eq.)	H	-0.15	0.94	0.27
114 (mj.)	H	-0.51	0.98	0.12
119 (mj.)	H	-0.21	0.97	0.47
	H	-0.11	0.98	0.64
- Populations				
172	H	-0.27	1	0.26
	L	-0.47	0.97	0.24
Alameda	H	-0.30	0.98	0.20
	L	- .34	0.97	0.38
- F_2				
172 x 171	H	-0.41	0.95	0.11
	L	-0.22	0.98	0.47
119 x 114	H	-0.66	0.97	0.19
119 x 26	H	-0.36	0.98	0.25
26 x 114	H	-0.31	0.97	0.04

teractions between level of infestation and host response etc. To clarify the genetics of the Orobanche attack, it will be necessary to dessign experiments handling with the lowest number of variates, having in mind that the strong environmental influence will always be present.

B2) O.crenata/plant - seeds/pod.

Almost general lack of significance in all the levels of infestation and for all types of materials. Most frequent values range from -0.30 to + 0.25, clearly indicating a distribution around zero. Partial correlation coefficients are also generally non significant.

B3) Seeds/pod - seeds/plant and pods/plant.

Total correlation coefficients are some times significant but in most of the cases they are not. That is: any number of, for example, seeds per plant can be expected given a certain number of seeds per pod.

But the study of the partial correlation shows a different aspect. When the effects of pods/plant and parasites/plant are removed, seeds/pod is strongly and positively correlated with seeds/plant. Current values of the coefficient are 0.60 to 0.90. This is a logical result: for fixed numbers of pods and parasites per plant, the total number of seeds will depend on the number of seeds per pod. A similar fact is logically found for the correlation between seeds per pod and pods per plant when the effects of seeds and broomrapes per plant are removed: strong negative significant values of the coefficient are found, generally ranging from -0.60 to -0.90.

In all these cases, the kind (i.e., parentals, F_2, populations) and origin of the materials are independent of the results.

It is now easily understandable why the total correlation coefficient between seeds/pod and both seeds and pods/plant are distributed around zero: for a fixed value of seeds/plant, seeds/pod and pods/plant are negatively correlated; for a fixed value of pods per plant, the other two characteristics are positively (and with the same order of magnitude) correlated. The total correlation coefficient is cancelled out.

C) Multiple regression with standardized values.

Expressions of the form

$$y = k_1x_1 + k_2x_2 + k_3x_3$$

are obtained, where y (seeds/plant in our case) and x_i (independient variables) are referred to mean zero and standard deviation unity. The interest of these expressions lies in the fact that if k_1 is twice the value of k_2, the variable x_1 is affecting the y value in a order of magnitude double than that of x_2. Coefficients k_i are currently mentioned as path coefficients.

Let's x_1, x_2 and x_3 stand for broomrapes/plant, pods/plant and seeds/pod respectively (standardized variables). Table 2 contains some values of

the corresponding k_i.

It is obvious that pods/plant is the most important of the three characters related to seeds/plant its magnitude being roughly speaking, triple in absolute value than each one of the two others. Number of O. crenata per host plant is, as expected, affecting negatively the final effect and seeds/pod positively (the effect of seeds/pod is much more variable than that of parasites per plant). It can be seen that the different level of infestation is not producing any discerning pattern in the cases studied.

D) The causal scheme.

Even in a very approximate and rough way, we can fill the links between characteristics as shown in fig. 3. The low value obtained for the path coefficient of "unknown" causes means an almost complete determination of seeds/plant by the three characteristics studied. Of course this is a consequence of the strong correlation of the final effect with one of its inmediate causes pod/plant. In other words, we have transferred the problem to the determination of pods/plant.

In terms of the effect of the Orobanche attack, it is interesting to observe that its action on seeds/plant is through pods/plant and not through seeds/pod. Next step in this study, once the possibilities of the methology has been tested, will be to split pods/plant in its components (number of stems, number of nodes per stem, pods per node etc). To see if Orobanche is affecting the final effect through a preferential way as it is the case here analyzed. To analyze the effect on yield a new variable will be necessary: the seed size, whose strong well known effect on yield could mask most of the other causes. The use of partial correlations and of multiple linear regression will be again very convenient.

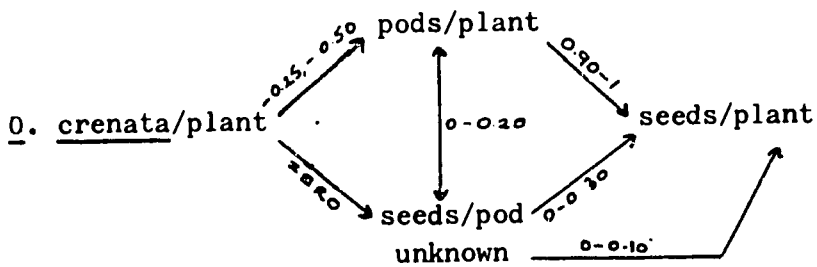


Fig. 3

Causal relationships

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RESISTANCE TO OROBANCHE CRENATA FORSSK. IN VICIA SATIVA L. II. CHARACTERIZATION AND GENETICS

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Abstract. Resistance of common vetch (Vicia sativa L.) to broomrape (Orobanche crenata Forssk.) is characterized and studied from a genetic point of view. The best index of resistance is the number of O. crenata per host plant, the average size of the parasite being less consistent. No dominance, or perhaps a very slight partial dominance for resistance, is suggested by the results. The study of segregating generations shows no apparent genic association between earliness of flowering and resistance to Orobanche, despite the results obtained from germplasm collections.

INTRODUCTION

Orobanche crenata Forssk. is a common parasitic weed that causes great damage to many crops, e.g. faba beans, peas, lentils. It also attacks common vetch, Vicia sativa L., a forage crop of mediterranean areas. The attack on this crop is not very serious, and selection for resistance proved to be useful (Gil, Martin and Cubero, 1982). This resistance can be used improve commercial cultivars and also as a model to study the genetics of resistance in the common vetch relatives mentioned above which are more important economically.

This paper is the first report on the characterization of Vicia sativa resistance to O. crenata.

METHODS AND MATERIAL

Two commercial cultivars, Prussia and VA74, were selected as susceptible parents, and two advanced lines, AO-1 and VS473A, as resistant ones. Pots were filled with 1.5 kg. of sterilized sand mixed with parasite seeds; two loses were used: 0.125 and 0.025 g/pot of parasite seeds. Three plants of vetch were sown in every pot. Observations were made carefully each week for the presence of parasitic plants attached to the roots, starting three months after sowing. Even those at the earliest stages of development were checked under a microscope. Number, size and weight were recorded in each case.

Crosses were made between the resistant lines 485B, 486A and 417B, and the susceptible lines CS-50, P (from Prussia) and VA (from VA74) by

standard methods. The behaviour of parents, and their F1, F2, F3 and backcrosses in both directions were studied by sowing all this material in a very heavily infested field. The seeds were sown separately and the design was randomised. Four different crosses and their progenies were studied. The number of mature plants checked ranged from 31 to 36 for parental generations, 21 to 36 for F1, 15 to 36 for backcrosses (only one case with 5), 120 r F2 and 12 each of 24 to 30 F3 families per cross. Results were scored per individual mature plant. Comparisons between mean values were performed by transforming individual data into log (x + 0.5), x being the number of broomrapes per host plant (Cubero, 1983).

RESULTS AND DISCUSSION

Differential behaviour of resistant and susceptible lines

Fig. 1 shows the total number of O. crenata per pot for each of the four lines studied. Resistant lines had much fewer parasites at both dose levels. The behaviour of the resistant line 473A is particularly interesting because the total number of Orobanche attached to the roots seems quite independent of the parasite density in the soil: the resistance is the same in both cases.

A decline in the total number of attached parasites after one to several weeks is most apparent in the case of the higher degree of infestation. This effect can be explained by the lack of vigour of the host plants (not producing further germination stimulants), or by competition between attached and germinating broomrapes or, finally, because of damage to the root system by established parasites.

Fig. 2 and table 1 give the average size of the Orobanche. In susceptible lines (VA and Prussia) the growth rate slows with heavy infestation after four weeks (Fig. 2b) as would be expected because of the greater competition between the parasite plants. In resistant lines the growth rate shows a much more irregular pattern. A possible explanation could be a less intense competition between the parasites giving a more random effect.

This random effect may also be the cause of the strange behaviour of the most resistant line, 473A. Here maximum average heights were 17.7 and 23.5 mm respectively at low and high levels of infestation, but based in the first case on only 7 parasite plants (ranging from 1 to 45 mm) and in the second on 6 parasite plants, one of which was the tallest in the whole experiment, producing the strange peak in fig. 2b. By comparison the susceptible line Prussia had 32 parasites averaging 14.6 mm at low infestation and 155 parasites at the high level of infestation. The same random effect may be the cause of the low value recorded in the last week as only three parasite plants were found in the pot.

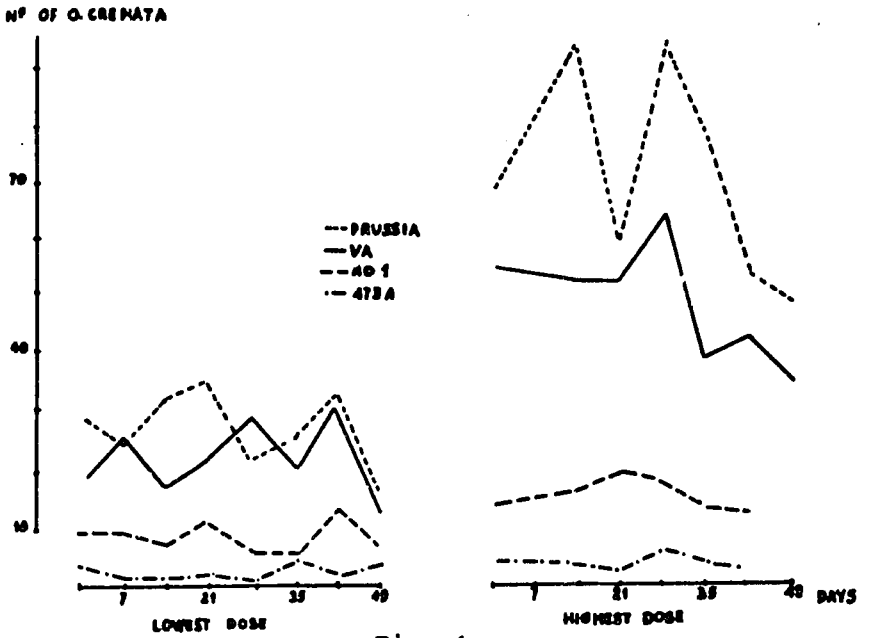

Fig. 1

Number of O. crenata on resistant and susceptible lines of Vicia sativa at two levels of infection.

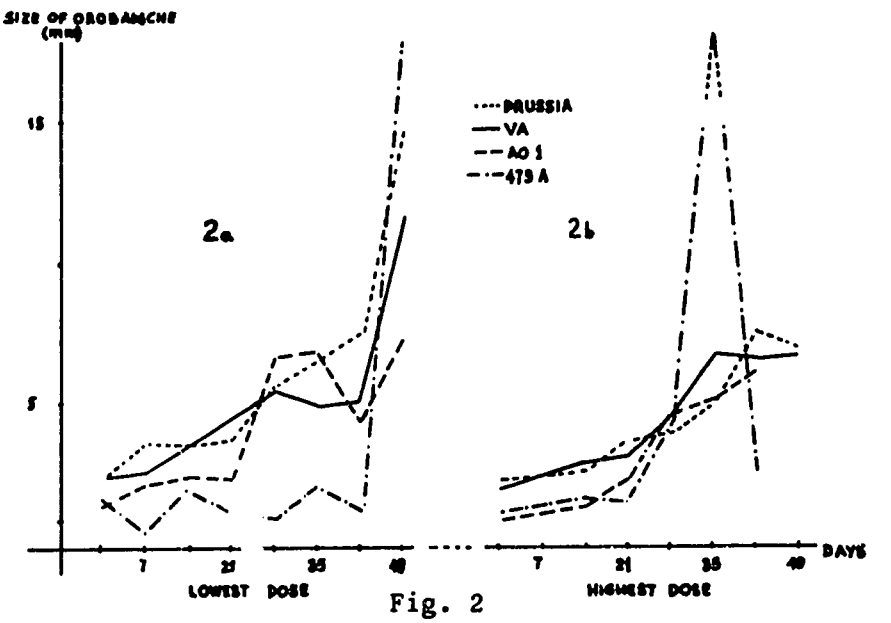

Fig. 2

Average size of O. crenata on different lines of Vicia sativa at two levels of infection.

TABLE 1

Growth of *O. crenata* on resistant and susceptible lines.

Date (1)	Number (2)	473 A Length (mm) $\bar{X}$	473 A Length (mm) Range	AO.1 Number	AO.1 Length (mm) $\bar{X}$	AO.1 Length (mm) Range	PRUSSIA Number	PRUSSIA Length (mm) $\bar{X}$	PRUSSIA Length (mm) Range	VA - 74 Number	VA - 74 Length (mm) $\bar{X}$	VA - 74 Length (mm) Range
Lower dose												
0	7	1.6	0.5-4	19	1.5	0.5-4	56	2.6	0.5-7	36	2.6	0.5-8
7	2	0.5	0.5	19	2.2	0.5-8	48	3.7	0.5-11	51	2.8	0.5-9
14	1	2	2	14	2.4	0.5-19	62	3.7	0.5-10	35	3.6	0.5-7
21	4	1.2	0.5-3	22	2.5	0.5-8	70	3.9	0.5-10	44	4.6	0.5-10
28	1	1	1	12	6.8	1 -23	43	5.6	1 -18	57	5.5	1 -17
35	9	2.2	0.5-7	11	6.9	0.5-20	50	6.7	1 -24	41	5.0	1 -20
42	3	1.3	1 -2	26	4.5	0.5-19	65	7.6	1 -47	61	5.3	1 -24
49	7	17.7	1 -45	12	7.5	1 -50	32	14.6	1 -47	26	11.7	1 -33
Higher dose												
0	7	1.3	0.5-2	27	1.0	0.5-3	137	2.4	0.5-10	110	2.2	0.5-7
7		Not recorded										
14	6	1.6	1 -3	32	1.6	0.5-4	188	2.7	0.5-9	105	3.0	0.5-11
21	4	1.6	0.5-3	38	2.4	0.5-12	118	3.8	1 -11	105	3.3	0.5-15
28	11	4.4	0.5-20	35	4.7	0.5-17	190	4.2	0.5-20	129	4.5	0.5-22
35	6	23.5	1 -83	25	5.2	1 -29	155	5.1	0.5-22	77	7.0	1 -33
42	3	2.7	2 -4	23	6.3	0.5-35	108	7.7	1 -38	85	6.8	0.5-44
49		Not recorded										

(1) Days from sowing on 2/March/82.

(2) Number of *Orobanche* of any size attached to the host rost system.

To see if susceptible and resistant lines differ in the time taken for attachment of the parasite to the roots a new experiment was designed and performed under exactly the same conditions. Plants were checked weekly, starting from 36 days after sowing. Two small broomrapes were found on Prussia 84 days after sowing, and 4 days later on the other three lines. Thus it seems that there are no differences in the time for attachment (and very probably for germination) of the Orobanche.

The following conclusions were reached.

(1) There is a mechanism of resistance to O. crenata in Vicia sativa. Resistant lines are not immune but show very few parasitic tubercles on the roots in pot conditions and practically no broomrape shoots under field conditions after three years.

(2) There are no differences between susceptible and resistant lines in the time taken to make attachments.

(3) Differences between susceptible and resistant lines are absolutely clear concerning the number of parasites attached to the roots (Fig. 1). This is, therefore, the best measure of resistance.

Genetics of resistance

Parents, F_1, F_2, F_3 and backcrosses $P_1 \times F_1$ and $P_2 \times F_1$ were studied. Figs 3 to 6 show the results for all but the F_3. The four crosses show an identical pattern. The susceptible parents show not only a greater number of broomrapes per host plant but also a higher range of variation. F_1 showed intermediate values between parents, and backcrosses showed the same pattern between F_1 and the respective parent. F_2 showed a greater variation, as expected, and their mean values were close to those of their F_1's (bearning in mind that to compare mean values a log transformation was performed).

Fig. 7 shows the results obtained for the F_3 in the four crosses. Horizontal and vertical coordinates represent mean values and frequency of F_3 families respectivaly. The absence of more than one mode (the results do not allow to admit bimodality in the case of 485B x CS-50) confirm the same fact in the F_2 and suggest the absence of major genes controlling the resistance of V. sativa to O. crenata. Rather, a quantitative (i.e. polygenic) system is strongly suggested by the sequence of values obtained for each generation compared with their parental generations. As can be seen in figs 3 to 7 the mean values are increasing from F_1 to F_3 (the case of VA x 458B, with an F_2 value slightly less than that of its F_1, could be due to sampling effects), but this increase never reaches the mid-point between the parental lines. If transformed data are used, the mid-point is reached by the F_3. All this suggests the presence of a strong additive system, producing almost intermediate inheritance (i.e., no dominance), perhaps accompanied by weak partial dominance for resistance. A very similar system has

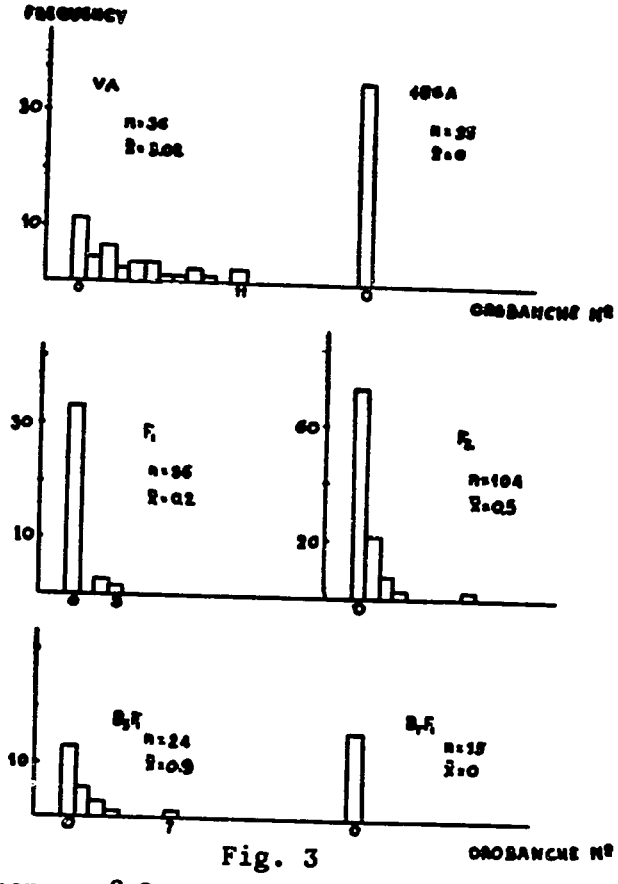

Fig. 3

Frequency of O. crenata ocurrence on parental, F_1 and segregating generations of the cross VA x 486A lines of Vicia sativa.

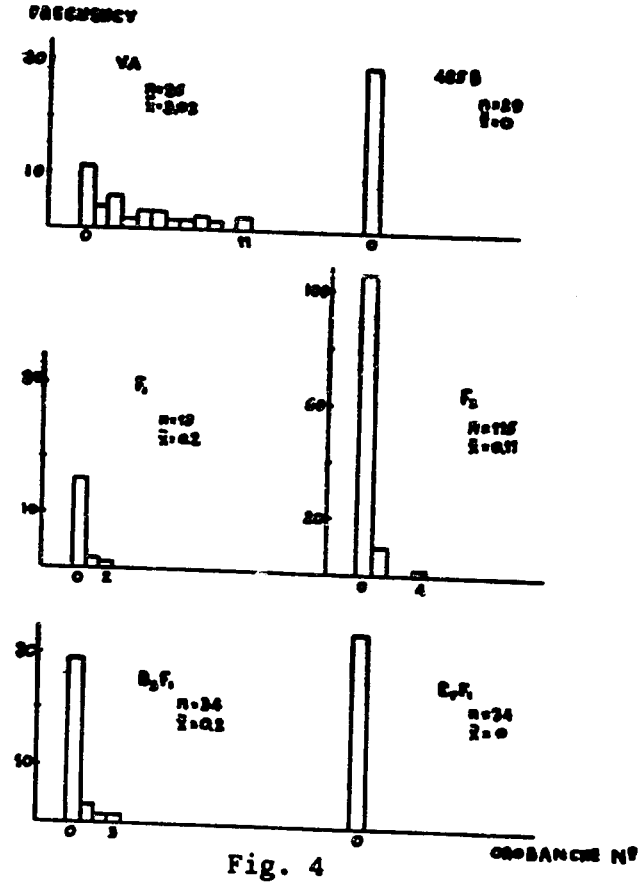

Fig. 4

Frequency of O. crenata ocurrence on parental, F_1 and segregating generations of the cross Va x 485B lines of Vicia sativa.

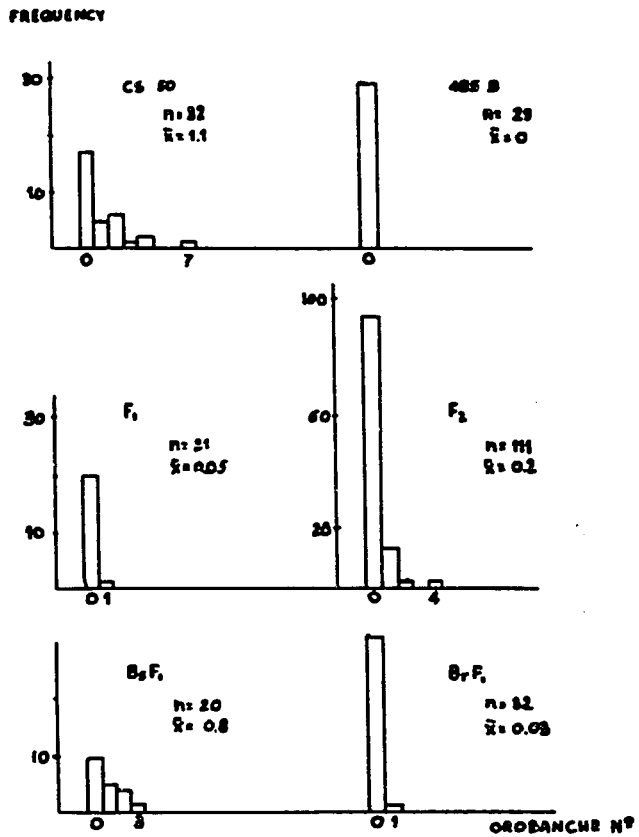

Fig. 5

Frequency of O. crenata ocurrence on parental, F_1 and segregating generation of the cross CS50 x 485B lines of Vicia sativa.

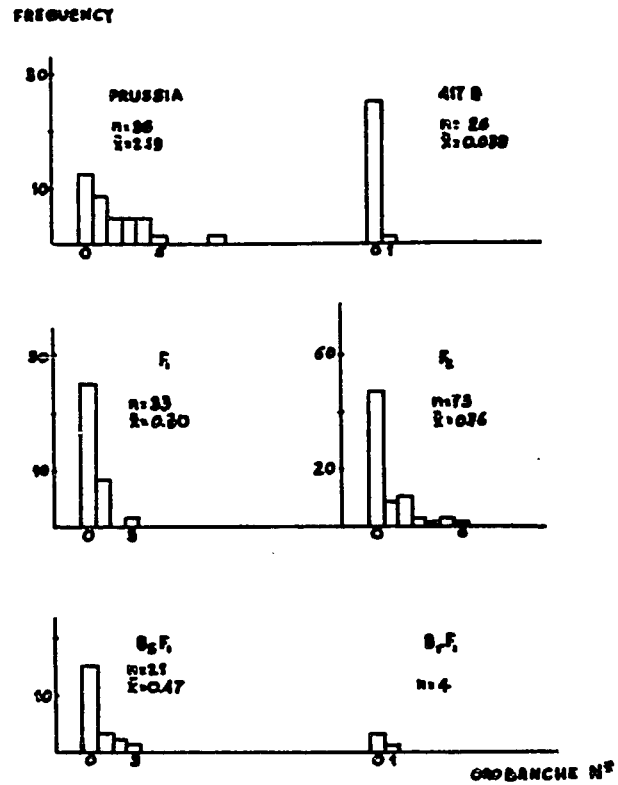

Fig. 6

Frequency of O. crenata ocurrence on parental, F_1 and segregating generation of the cross Prussia x 417B lines of Vicia sativa.

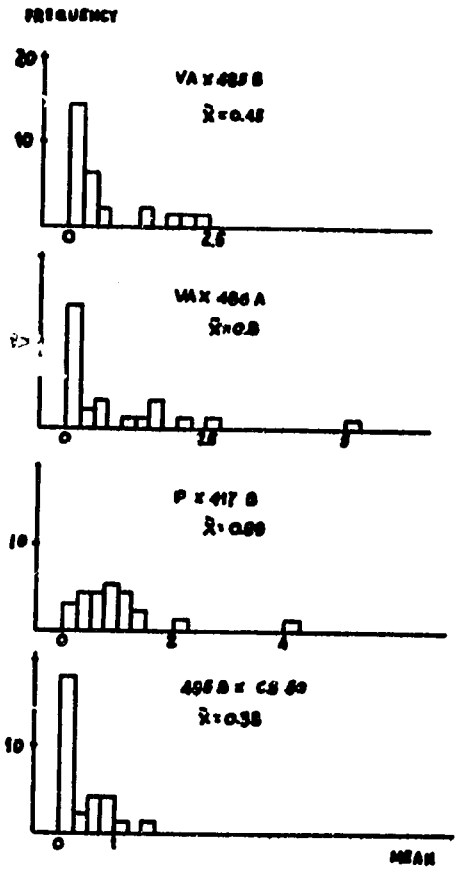

Fig. 7

Frequency of ocurrence of mean number of O. crenata on F_3 and crosses between resistant and susceptible lines of Vicia sativa.

also been proposed for V. faba (Suso, 1980; Cubero and Martinez, 1980).

Previous studies (Gil, Martin and Cubero, 1982) have shown a significant correlation coefficient between earliness flowering and resistance to O. crenata in V. sativa. This correlation was obtained from studying a large collection of crop accessions. Using our F_2 (i.e. segregating material) we have tested the hypothesis of genic association between both characteristics. F_2 plants were classified into three groups of earliness and a X^2 test for association was performed with no positive results. Thus, it seems that genes for precocity and genes for resistance are not linked. The correlation found in our collection is probably due to the fact that the earliest accessions came from mediterranean regions where Orobanche is endemic.

The following conclussions seem justified.

(1) The genes controlling resistance to O. crenata in V. sativa seem to show no dominance or, if any, a very low level of partial dominance for resistance.

(2) When segregating material (F_2) is studied, there is no genetic association between earliness in flowering and resistance to broomrape, in spite of results obtained from studying a germplasm collection. The latter results coul be due to natural and/or artificial selection in regions where the parasite is endemic.

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COMPARATIVE STUDIES ON HERBICIDES FOR OROBANCHE CONTROL IN TOMATO+

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Abstract. Three soil-applied herbicides chlorthal-dimethyl (DCPA), diphenamid and trifluralin alone and in combinations and the foliar-applied herbicide, glyphosate, were tested in the greenhouse for the control of *Orobanche ramosa* L. in tomato (*Lycopersicum esculentum* Mill). Trifluralin and diphenamid mixtures at different rates were ineffective against *Orobanche*. Diphenamid at 8 kg a.i./ha, DCPA at 5 and 10 kg a.i./ha, DCPA plus diphenamid at 5+6 kg a.i./ha and glyphosate at 0.04 + 0.03 + 0.03 kg a.i./ha, applied sequentially, caused significant reduction in *Orobanche* dry weight; however, all these treatments were phytotoxic to tomato.

INTRODUCTION

Broomrapes (*Orobanche* spp.) are noxious root-parasitic weeds which attack many broadleaf crops, particularly of the Solanaceae and Fabaceae. Soil fumigation with methyl bromide and chloropicrin, and hand-pulling are the most commonly used control measures in Lebanon (Saghir and Dastgheib, 1978). Worldwide, several measures have been investigated for the control of broomrape. These include biological, chemical, cultural and mechanical methods, in addition to the use of resistant crop varieties (Wilhelm *et al.*, 1958; Zahran, 1970; Kasasian and Parker, 1971; Cubero, 1973; Saghir *et al.*, 1973; Abu Gharbieh *et al.*, 1978; Basler and Haddad, 1979).

Promising results have been achieved in experiments on the control of *Orobanche* using synthetic germination stimulants, which induce parasite seed germination in the absence of the host (Johnson *et al.*, 1976; Saghir, 1978; Saghir, 1979; Saghir *et al.*, 1980). Several herbicides including bentazone, butylate, diphenamid, glyphosate and trifluralin have been tested for control with varying degrees of success (kasasian, 1973; Bischof and Koch, 1974; Puzzilli, 1976; Saghir, 1979; Jacobsohn and Kelman, 1980).

METHODS AND MATERIALS

Three experiments were conducted in the greenhouse using clay pots, 25 cm in diameter, filled with a soil-peatmoss mixture. Sixty mg of *Orobanche ramosa* L. seeds were mixed thoroughly with the soil mixture in each pot, exceptfor the non-inoculated control pots.

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Trifluralin and diphenamid were applied in sequence at 0.25 + 4 kg/ha and 0.5 + 2 kg/ha. In the first experiment, trifluralin was incorporated with the top 5 cm of soil in the pot, after which diphenamid was sprayed on the soil surface. Four weeks-old tomato seedlings of the local cultivar "Marmande" were transplanted into the pots five days after treatment. Two sublethal rates of glyphosate, 0.025 and 0.05 kg/ha, were used in a spray volume of 500 L/ha. Application of the herbicide was made 17 days after transplating, when tomato root exudates are most active, and Orobanche seed germination would have started (Ballard et al., 1978).

In a second experiment, trifluralin and diphenamid were again used at 0.5 + 2 and 0.25 + 4 kg/ha. The required amounts of the two herbicides were dissolved in 600 c.c of water and drenched into the pots. Glyphosate was sprayed at 0.06, 0.08 and 0.06 + 0.06 kg/ha; the first application made was 17 days after tomato transplanting, and the second application one week later.

A third study was conducted using DCPA at 5 and 10 kg/ha, diphenamid at 8 kg/ha, and a mixture of DCPA and diphenamid at 5 + 6 kg/ha. All herbicides were used as drench applications in 600 c.c of water/pot. Glyphosate was sprayed at 0.05 + 0.05, 0.04 + 0.03 + 0.03, and 0.1 kg/ha as foliar applications in 500 L of water/ha. All treatments were made five weeks after transplanting, and the second and third applications, one and two weeks later. All doses of herbicides are expressed in terms of active ingredient (a.i). All experiments were replicated five times in a completely randomized design.

RESULTS AND DISCUSSION

The result of the first experiment indicated that trifluralin and diphenamid at 0.25 + 4 kg/ha failed to prevent Orobanche parasitism on tomato (Table 1.). This contradicts the findings of Dastgheib (1974, pp 65-69) who reported that drench applications of trifluralin and diphenamid at 0.25 + 4 and 0.5 + 6 kg/ha reduced significantly the dry weight and and number of shoots of O.ramosa in tomato.

Data presented in Table 2 show that the number of shoots and the dry weight of Orobanche were unaffected by any of the herbicides at the rates tested. Glyphosate was phytotoxic to tomato when used at 0.08 and 0.06 + 0.06 kg/ha and resulted in significant reductions in tomato shoot dry weight and fruit yield.

All herbicide treatments in the third experiment (Table 3.) failed to reduce the number of Orobanche haustoria. However, DCPA and diphenamid applied alone or in combination at different rates, as well as glyphosate at 0.04 + 0.03 + 0.03 kg/ha caused significant reductions in the dry weight of Orobanche, but not in the number of haustoria, as compared to the inoculated control.

Initial haustorial development was not affected by the herbicides tested; however, these herbicides inhibited shoot development, since with

time, enough of the herbicides had been taken up and accumulated in the parasite. This could explain the decrease in Orobanche shoot dry weight, without causing an effect on the number of haustoria. All herbicides caused significant reductions in tomato shoot dry weight, as compared to the non-inoculated control.

It may be suggested that glyphosate should be tested at lower sublethal doses. Several sequential applications should be used since the herbicide was able to reduce O.ramosa infestation, but was phytotoxic to the crop.

Table 1. Effects of trifluralin plus diphenamid and glyphosate on O.ramosa in tomato.

Treatment	Kg/ha (a.i.)	Orobanche ramosa		Tomato	
		Average no. of shoots/ pot	Dry weight g/pot	Shoot dry Weight g/plant	Fruit yield g/plant
Trifluralin + diphenamid	0.25+4	14.7	6.0	37.8	649.0
Trifluralin + diphenamid	0.5+2	10.3	6.1	42.3	597.8
Glyphosate	0.025	11.3	5.5	37.0	606.2
Glyphosate	0.05	11.6	5.1	40.8	585.3
Control + Orobanche	-	14.8	5.0	36.2	594.5
Control - Orobanche	-	-	-	40.3	527.3
		N.S.	N.S.	N.S.	N.S.

Table 2. Effects of trifluralin plus diphenamid and glyphosate on *Orobanche* in tomato.

Treatment	Kg/ha (a.i.)	*Orobanche ramosa*		Tomato	
		Average no. of shoots/ pot	Dry Weight g/pot	Shoot dry weight g/plant	Fruit yield g/plant
Trifluralin + diphenamid	0.25+4	4.0	3.2	23.8 abc*	65.2 ab*
Trifluralin + diphenamid	0.5+2	4.0	5.2	23.1 abc	46.8 b
Glyphosate	0.06	3.6	3.9	32.0 ab	48.8 b
Glyphosate	0.08	3.8	4.2	21.1 c	40.0 b
Glyphosate	0.06+0.06	4.0	5.4	21.4 bc	9.4 b
Control + *Orobanche*		5.0	4.7	19.8 c	17.4 b
Control - *Orobanche*		-	-	33.8 a	123.0 a
		N.S.	N.S.		

* Values with a common letter within columns are not significantly different according to Duncan's multiple range test at 5% level of significance, N.S. = Not significant.

Table 3. Effects of DCPA, diphenamid, and glyphosate on *Orobanche ramosa* in tomato

Treatment	Kg/ha (a.i.)	*Orobanche ramosa*		Tomato	
		Average no. of haustoria pot	Dry weight g/pot	Shoot dry weight g/plant	Fruit yield g/plant
DCPA	5	23.6	4.02 bc*	29.8 b*	144.6
DCPA	10	11.8	2.94 bc	30.9 b	131.2
Diphenamid	8	23.2	2.10 c	33.9 b	142.0
DCPA + diphenamid	5 + 6	16.4	3.74 bc	35.4 b	90.8
Glyphosate	0.05+0.05	14.6	5.40 ac	29.6 b	100.0
Glyphosate	0.04+0.03+0.03	16.6	2.74 bc	32.5 b	96.8
Glyphosate	0.1	25.0	5.76 ab	29.7 b	147.6
Control + *Orobanche*	-	24.0	8.2 a	29.9 b	101.4
Control - *Orobanche*	-	-	-	44.4 a	107.8
		N.S.			N.S.

* Values with a common letter within columns are not significantly different according to Duncan's multiple range test at 5% level of significance, N.S. = Not significant.

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Third International Symposium on Parasitic Weeds, Aleppo, 1984

CRITICAL EVALUATION OF CONTROL METHODS FOR OROBANCHE RAMOSA L. OCCURING IN SMALLHOLDER VEGETABLE FARMS OF THE KHARTOUM PROVINCE, SUDAN

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Abstract. For the management of broomrapes (Orobanche spp.) a variety of measures can be employed; their use is briefly discussed and evaluated under the aspect of suitability for smallholder vegetalbe farmers in the Khartoum Province. No completely satisfactory control method has been found so far, but preliminary trials with the postemergence application of glyphosate on eggplants, and the solar heating technique with polyethylene mulch showed promising results; the latter seems appropriate especially through its effectiveness and simple handling; additionally positive side-effects occur against annual weed species and Meloidogyne nematodes.

INTRODUCTION

Hemp or branched broomrape Orobanche ramosa L. was found during a survey in vegetable fields of Khartoum Province, Sudan, winter to early summer 1982 but not in Rahad or Gezira Schemes. It commonly parasitizes tomatoes, eggplants and carrots on Nile-alluvial soil, often with devastating effects. Less frequently it attacks cabbages, potatoes and the weeds Chrozophora plicata (Vahl) A. Juss ex Spreng (Euphorbiaceae), Solanum dubium Fresen. (Solanaceae) (Braun, 1982; Jung, 1983). Other potential hostplants growing in Khartoum Province as vegetables are cucumbers, squash, lettuce, cauliflower, peppers, and as weeds Malva parviflora L. (Malvaceae), Solanum nigrum L. (Solanaceae) (Abu-Irmaileh, 1979). Other Orobanche spp. occuring in Sudan without economic importance are: O. cernua var. desertorum (G. Beck) Stapf. in the Red Sea District and O. minor Sm. in Erkowit, Khartoum Province and the Immatong Mountains (Ismail, 1979; Musselman, 1984). Orobanche is not controlled regularly yet. Frequently, handpulling is used in severely infested fields. Other control measures are under investigation; first results are reported here.

CONTROL MEASURES - SITUATION IN SUDAN

Preventive and cultural control

From the variety of control measures falling into this category essentially only handpulling is used to any extent so far; but because of the incomplete destruction of all emerging Orobanche shoots, its effectiveness is questionable. Although nitrogen (Ca-cyanamide, ammoniumsulphate) reportedly reduces the incidence and vigor of infestations in tomatoes and broadbeans (Vicia faba L.) significantly, the usually quite high urea applications in vegetable crops in Khartoum Province obviously did not reduce O. ramosa parasitism sufficiently. NPK-fertilizers and Ca-cyanamide are not yet available to farmers in the area. Prospects for trap-crops have not been evaluated so far but should be tried. On the other hand, the non-cropping of such important susceptible crops like tomatoes or eggplants during the winter season when the Orobanche parasitism occurs, obviously would cause considerable economic disadvantages to smallholder farmers.

Resistant cultivars

The breeding of crop cultivars resistant against Orobanche parasitism so far succeeded only for a few crops. In Sudan, for vegetable crops no resistant cultivars are available yet. Potentially, this would be a quite appropriate way for smallholders to keep Orobanche under control. Some limitations, however, might arise in mixed cropping, which is practised to some extent in the area, if a resistant and susceptible crop are grown together.

Biological control

Biological control with insects and fungi has been mainly tried in Eastern Europe. The successful use of predators in Khartoum Province seems to be problematic due to the short presence of living Orobanche shoots between mid-December and end of February. The production and distribution of fungus-material, if effective, has to be centrally organized for smallholder farmers to be economical and efficient.

Synthetic germination-stimulants

Experience with the strigol analogue GR 7 and the ethylene generating ethephone in stimulating seed germination of Striga hermonthica (Del.) Benth. is readily available in Sudan (Babiker and Hamdoun 1982, 1983). Since these chemicals also seem to be effective against Orobanche - if economically feasible - they could prove to be an efficient control method. But so far no large-scale field trials have been conducted.

Herbicides

From the different means of herbicide application only preliminary trials with sublethal doses of glyphosate in eggplants have been conducted so far (Table 1). 40 g a.i./ha sprayed 17 days after transplanting increased fruityield of eggplants infested with O. ramosa above the untreated controls by 18 %, while 80 and 160 g a.i./ha reduced the yields significantly. Since there is only a narrow span of beneficial effects on the parasitized crop and its possible kill this narrow span can only be achieved by applying the accurate dosage as uniformly as possible. Because of the quite insufficient application methods presently used by small farmers (Walter et al.,1983), this control measure seems not very practicable yet, if farmers have to do it themselves. Treatments carried out by licensed applicators, however, could probably solve this problem.

Table 1: Effects of Orobanche ramosa L. and application of different doses of glyphosate on yield of eggplants cv. "black beauty"

	Yield (kg/ha)	Compare (%) to 1.	to 2.	Number Orob. plants/plot	(%) infestation
1. no Orob., no gly	5361.9	-	+10.4	-	-
2. Orob., no gly	4857.1	- 9.4	-	26	43
3. Orob., +40 g gly/ha	5752.4	+ 7.3	+18.4	24	42
4. Orob., +80 g gly/ha	4466.7*	-16.6	- 9.0	20	33
5. Orob., + 160 g gly/ha	4238.1*	-20.9	-12.7	19	32

*) significantly different from 3 at 5 % level

Soil sterilization

Common soil sterilization techniques are not applicable from an economical point of view on a large-scale basis. Also flooding of fields resulting in anaerobic conditions, toxic to many organisms, is obviously not sufficient in reducing the Orobanche problem in the area.

A more practicable approach for arid and semi-arid regions has been made through solar heat sterilization of pre-irrigated fields covered with clear polyethylene mulch during the summer (Katan et al., 1979; Jacobsohn et al., 1980). Preliminary trials in the Khartoum Province resulted in a complete control of a light to medium infestation with O. ramosa, a 80 % reduction in the number of annual weed species and a significant decrease in the visible attack of Meloidogyne sp. on roots of eggplants grown as bioindicators, already after 30 days of covering. The cost for mulching material is considerable especially in developing countries. The efficiency however, can be increased through repeated use on several areas during the hot season and in subsequent years. Additionally the positive side effects and the simple handling make this method especially suited for intensive vegetable production, as in the Khartoum Province, where nematodes also play an important role (Yassin,1979).

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EFFECT OF PLANTING FLAX ON THE SUBSEQUENT INFESTATION OF TOMATO BY OROBANCHE RAMOSA

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Abstract. Greenhouse pot experiments were conducted to study the potential of flax (Linum usitatissimum L.)and other crops, as trap crops for the branched broomrape (Orobanche ramosa L.) infesting tomato (Lycopersicon esculentum Mill.); and , to study the effect of time of removal of flax on parasitism in the subsequently planted tomato. The results indicated that flax has a great potential as a trap crop for O. ramosa infesting tomato. Also, planting flax for a period of 4 to 6 weeks prior to planting tomato can effectively reduce infestation in tomato .

INTRODUCTION

Tomato is the leading vegetable crop grown in Jordan (Steitieh et al., 1978). Tomato suffers to various degrees from broomrape (Orobanche ramosa L.) infestations in most growing areas. O. ramosa is the most widespread species of the genus Orobanche in Jordan (Abu-Irmaileh, 1979).It attacks most of the solanaceous crops, cucurbits, broadbeans (Vicia faba L.), and other economically important crops, as well as various weed species (Abu-Irmaileh, 1979). Heavy infestations seriously limit tomato production, especially when the plants are infested during their early stages of development (Abu-Irmaileh, 1982 a). Effective control methods are quite limited. Satisfactory control of O. crenata Forsk. on broadbeans has been acheived by applying glyphosate(Schluter et al., 1979 and Schmitt et al., 1979). However, this herbicide did not show sufficient selectivity on other crops(Jacobsohn et al., 1980). The fumigant, methyl bromide effectively controls this parasite, but is highly expensive (Gold et al., 1978). Biological control has been obtained with the insect Phytomyza orobanchiae and some strains of the fungus Fusarium (Musselman, 1980). The introduction of such bioagents is hindered by the high cost of research. Cultural means of broomrape control include the application of fertilizers (Abu-Irmaileh, 1982 a and Kasasian, 1973), handweeding(Abu-Irmaileh, 1982 b ; Gharib, 1973 and Kasasian, 1973), crop rotation (Abu-Irmaileh, 1982 b), and trap cropping (Musselman, 1980 and Pieterse, 1979). Trap crops for this parasite include pepper (Capsicum annuum L.), sesame(Sesamum indicum L.) (Musselman, 1980), and flax (Abu-Shakra et al., 1970). It is known that root diffusates of the trap crops enhance

Orobanche seed germination (Abu-Shakra, et al., 1970 and Musselman, 1980), but the germinated parasite seed normally fails to attach to the roots of these crops.

In this research, the trap crop flax was tested for its ability to reduce O. ramosa infestation on tomato.

MATERIALS AND METHODS

Pot experiments were conducted in the greenhouse with an average temperature of 28 C during the day and 12 C at night from September 1981 and 1982 until June of each of the following years. Clay pots, 30 cm in diameter, containing approximately 12 kg of potting soil mixture (50 % clay, 33 % sand, 17 % silt, pH 8.0) previously fumigated with methyl bromide were used. Approximately 20,000 (100 mg) O. ramosa seeds were mixed with 300 g sand, and then with the soil of each pot. Two experiments were begun in September 1981. The first experiment was conducted to compare flax with other crops as a trap crop. The experiment included eight treatments. The seeds of beans (Phaseolus vulgaris L.), eggplant (Solanum melongena L.), flax, pepper, tomato, sesame, and garlic bulblets (Allium sativum L.) were planted in the assigned pots. Each crop was planted in six pots. Upon germination, plants were thinned to 5 seedlings per pot except for flax pots which contained about 200 seedlings per pot. A set of six unplanted pots was included as a control. Treatment each consisted of 6 replicate pots were arranged in a randomized complete block design. The planted pots were irrigated at weekly intervals. The control pots were watered whenever the soil surface got dry. The plants were allowed to grow for two months. Then, the plants were removed from the pots and the potting soil in each pot was cleaned from the roots. Tomato seeds were planted in all pots including the controls after the removal of the previous crop plants. Tomato seedlings were thinned to one plant per pot 3 weeks after germination.

The second experiment was conducted to study the effect of the time of removal of flax plants on O. ramosa infestation on tomato. The experiment included six treatments. About 200 seeds of flax (1 g) were planted in each pot.After about 50 % germination, flax seedlings were allowed to grow for either 2, 3, 4, 5 weeks, or to flowering (8 weeks). A set of six unplanted pots were included as a control. The treatments replicated 6 times were arranged in a randomized complete block design. Flax plants were removed after the assigned growing period. The potting soil was cleaned from the roots, then returned to the pots. Tomato seeds were planted in all pots including the control treatment, after the removal of flax plants from the last treatment,flowering,

i.e, 8 weeks. Tomato seedlings were thinned to one plant per pot 3 weeks after germination. This experiment was repeated during September 1982 until June 1983. The latter experiment included seven treatments; the removal of flax plants at 2, 3, 4, 5, 6, and 8 weeks (flowering) after germination, plus a control. All treatments were replicated six times, and arranged in a randomized complete block design. Data on tomato yield, tomato root and shoot dry weights, and the dry weight of the whole O. ramosa plants in each pot were recorded. The data were subjected to statistical analysis. The plant parts were dried in the oven at 70 C for a minimum of 48 hrs, or until their dry weights were stable.

RESULTS

The results of the various crops used in rotation with tomato indicated that only flax and pepper caused significant reduction in Orobanche parasitism on tomato as judged by the mean dry weight of the parasite plant (table 1). Eggplant,

Table 1

Effect of some crops grown before tomato on the dry weights of O. ramosa, tomato root and shoot and, tomato yield.

Treatment	Dry weights (g)			Tomato yield (g)
	O. ramosa [a]	Tomato		
		root	shoot	
Flax	.3 a	16.1	41.7	133
Sesame	17.0 bc	9.2	29.2	70
Beans	8.4 abc	4.6	23.6	96
Garlic	14.6 abc	5.4	20.4	32
Eggplant	12.0 abc	8.4	29.4	32
Pepper	4.1 ab	10.5	30.9	117
Tomato	8.9 abc	12.9	26.8	73
Control	20.7 c	5.6	30.9	36
		(N.S)	(N.S)	(N.S)

[a] Values within columns followed by a common letter are not significantly different at the 5 % level as determined by Duncan's multiple range test.

N.S= Values within column are not significantly different.

tomato, garlic, beans and sesame did not cause significant reduction of the O. ramosa dry weight, eventhough the values of the parasite dry weight were lower in these treatments than that of the control. Tomato yield and tomato root and shoot dry weights were not significantly affected by the various treatments.

The results of the effect of the time of removal of flax plants on the O. ramosa infestation on tomato indicated that flax can cause significant reduction of parasitism on tomato, if allowed to grow for 4 weeks in one experiment (table 2) or 6 weeks in another experiment (table 3). The values of the

Table 2

Effect of time of flax removal on O. ramosa dry weight infesting subsequently planted tomato, and on tomato root and shoot dry weights and tomato yield (Sep. 1981 - June 1982).

Treatment	Dry weights (g)			Tomato yield (g)
	O. ramosa[a]	Tomato		
		root	shoot	
Flax removal after				
2 weeks	15.1 b	6.6	35.8	88 b
3 weeks	6.1 ab	4.9	31.4	138 b
4 weeks	3.9 a	6.3	30.2	157 b
5 weeks	4.2 a	8.8	30.2	146 b
Flowering	4.6 a	6.0	29.8	90 b
Control	27.7 c	4.6	29.8	12 a
		(N.S)	(N.S)	

Table 3

Effect of time of flax removal on O. ramosa dry weight infesting subsequently planted tomato, and, on tomato root and shoot dry weights and tomato yield (Sep. 1982 - June 1983).

Treatment	Dry weights (g)			Tomato yield (g)
	O. ramosa[a]	Tomato		
		root	shoot	
Flax removal after				
2 weeks	31.3 b	3.6	33.4	24
3 weeks	18.4 ab	3.8	35.8	25
4 weeks	18.7 ab	4.1	39.0	60
5 weeks	11.1 ab	4.7	34.4	44
6 weeks	7.4 a	5.8	44.8	66
Flowering	4.8 a	5.7	45.0	34
Control	31.1 b	2.8	27.6	38
		(N.S)	(N.S)	(N.S)

[a] Values within columns followed by a common letter are not significantly different at the 5 % level as determined by Duncan's multiple range test.

N.S = Values within column are not significantly different.

O. ramosa dry weights were more reduced as the growing period of flax was extended. Tomato root and shoot dry weights were not statistically affected by the treatments. However, their values were the lowest in the control treatments (tables 2 and 3). Tomato yield was significantly the lowest in the control treatment in experiment 1 (table 2), but tomato yield was not affected by any treatment in experiment 2 (table 3). This experiment was, however, seriouly affected by the tomato yellow leaf curl virus infection.

DISCUSSION

In a previous study, flax and pepper caused 70 and 50 % control of O. ramosa on tomato, respectively(Abu-Irmaileh, 1982 b). In this research, the results indicated that flax and pepper can bring about 95 and 80 % control. This indicates that the results obtained are consistent in terms of causing significant reduction of O. ramosa infestation on the subsequently planted tomatoes. Sesame as a trap crop, did not show significant potential for reducing O. ramosa infestation, even though, it has been reported as a trap crop. (Musselman, 1980). Beans, eggplants and tomatoes as hosts for the parasite, and the nonhost crop, garlic, did not cause statistically significant reduction of parasitism on subsequently planted tomato. However, beans, tomato and garlic have been reported to be promising (Abu-Irmaileh, 1982 b).

The results of the effect of the time removal of flax on reducing parasitism on subsequently planted tomato indicated that growing flax for 4 to 6 weeks brought about significant reductions of O. ramosa dry weights. Flax root exudates are known to induce suicidal germination of the parasite seeds (Abu-Shakra, et al., 1970); thus, reducing the inoculum level in the soil. In spite of reduced infestation by O. ramosa there were no significant yield differences in the second experiment. This might has been due to the yellow leaf curl virus infection of the tomato plants in most treatments. In the first experiment, the yield of the untreated control treatment was significantly lower than other treatments (table 2).

The results of such experiments indicate that flax might be incorporated in agriculture as a trap crop for the broomrape O. ramosa. The period of planting flax for effective reduction of parasitism on subsequent tomato planting is short, i.e. 4 to 6 weeks. There would be no need for planting flax for longer periods, if flax is to be plowed under. However, field experiments should be conducted to verify the greenhouse results.

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INTEGRATED CULTURAL PRACTICES AND CHEMICAL CONTROL OF OROBANCHE CRENATA IN FABA BEAN

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Abstract, The effects of tillage practices, fertilization and chemical treatments were studied for control of Orobanche crenata Forsk. In faba bean (Vicia faba L.) in a field experiment conducted during 1982/83 season. Minimum and zero tillage increased crop yield significantly over conventional tillage without decreasing Orobanche infestation. Addition of nitrogen fertilizer had a significant positive effect on yield and tended to decrease Orobanche infestation.

Chemical control significantly reduced the number of Orobanche shoots as well as their dry weight, and increased yield of faba bean. The most successful treatment was glyphosate applied at 0.08 kg ai/ha 3 times at 15 days intervals beginning at the early flowering stage of faba bean. The second most successful treatment was glyphosate 2 times at the same rate but the second application with foliar fertilizer (Sandoflor). However, there was no significant difference in yield between these two treatments when combined with zero or minimum tillage.

INTRODUCTION

Orobanche species have been identified as important roots parasites of many legumes in the Mediterranean region and Middle East. Among all legume crops, faba bean (Vicia faba L.) appears to be the most sensitive to Orobanche crenata Forsk. The parasite spikes appear during the growing season especially during flowering, (Pieterse 1979; Saghir et al. 1978). The crop may be totally destroyed depending upon the level of the infestation of Orobanche seeds in the soil (Schmitt et al. 1979). There is no consistent practice that is helpful in reducing or eradicating this pest (Saghir et al. 1878).

The most effective prevention of Orobanche parasitism would be by growing resistant cultivars. Existing cultivars show differential susceptibility to the various Orobanche species and selection of resistant lines which could be used in breeding is in progress (Cubero 1973; ICARDA, 1981, 1982).

An attempt has been made towards biological control of O. crenata by Phytomyza orobanchiae. This fly attacks O. crenata, producing infestation of up to 90% of the fruits. The percentage of damaged seed capsuls can be high and very promising (Nemli et al. 1983).

Cultural practices for controlling this parasite are limited and not always effective. Some reports indicate that application of fertilizers and deep plughing are helpful in reducing Orobanche infestation (Aleksiev, 1967; Kasasian 1973).

Several investigations on chemical control of Orobanche have been undertaken. Research in the past has revealed that although most of herbicides appeared to be useless, some had a good effect. The most promising herbicides are glyphosate, propyzamide and trifluralin (Piglionica, 1975; Schmitt et al., 1979; ICARDA 1981, 1982, Zahran 1982). In order to achieve maximum control of O. crenata in faba bean, an integrated system involving both cultural and chemical control has been under study in field experiments at ICARDA. This paper reports the results of the first year of this study.

MATERIALS AND METHODS

The experiment was conducted in 1982/83 season at ICARDA experimental sub-site at Kafr Antoon in northern Syria using a field with natural infestation of O. crenata. The cultivar of faba bean was ILB-1814 (Syrian Local Large) which is highly susceptible to Orobanche. The trial was laid out in a split-split plot design with three tillage systems as main plots, two fertilizer levels as sub-plots and ten chemical treatments in sub-sub plots. There were four replications. Faba bean was planted on November 30 manually in rows 50 cm apart. Six rows of 5 m lenght each constituted a plot. Fertilizer was applied at planting time all plots had 60 kg phosphorus per ha (as P_2O_5), half the plots had additional nitrogen at 40 kg/ha (as NH_4NO_3) Chemicals were applied in the same direction as faba bean rows using a pressured knapsack sprayer and 400 l/ha spray solution. The timing of application was based on the phenological development of faba bean. The number of Orobanche shoots was counted just before physiological maturity and harvested for their dry weight.

RESULTS

Tillage system significantly affected the yield of faba bean. The highest yield was obtained with minimum and zero tillage. Significantly lower numbers of Orobanche shoots and total dry weight (TDW) of Orobanche were observed on conventional tillage treatment (Table 1).

Table 1. Effect of tillage system on yield of faba bean and Orobanche

Tillage System	Yield kg/ha	Number of Orobanche per m²	Total Dry Weight of Orobanche g/m²
Conventional (deep)	1172.7	41.9	36.9
Minimum (shallow)	1572.7	50.2	51.0
Zero	1514.4	43.3	41.8
LSD at 5%	375.0	6.2	12.3

Addition of nitrogen fertilizer had a significant effect on faba bean yield. Number and total dry weight (TDW) of Orobanche was lower in this treatment, but the difference was not statistically significant (Table 2).

Table 2. Effect of fertilizers on yield of faba bean and Orobanche control.

Nitrogen Treatments	Yield kg/ha	Number of Orobanche per m²	Total Dry Weight of Orobanche g/m²
nil	1322.9	48.4	45.6
40 kg N/ha	1517.7	41.8	40.9
LSD	224.4	N.S.	N.S.

The best chemical treatment for controlling Orobanche was application of glyphosate, at 0.08 kg ai/ha at 15 days interval, three times, starting at the beginning of flowering in faba bean. The second best treatment was the application of glyphosate twice at the same rate with a 15 days interval. This significant decrease of Orobanche shoots per unit area and their dry weight resulted in the increase in the yield of faba beans under these chemical treatments (Table 3).

Table 3. Effect of chemical application on yield of faba bean and Orobanche control.

Chemical Treatments	Yield kg/ha	No.of Orobanche per m²	Total Dry Weight of Orobanche g/m²
1 Untreated	1256.3	80.4	58.7
2 Glyphosate at 0.08 kg ai/ha	1358.4	57.5	46.7
3 Glyphosate at 0.08 kg ai/ha (2 applications)	2085.1	11.2	8.3
4 Glyphosate at 0.08 kg ai/ha (3 applications)	2140.8	4.7	4.2
5 Propyzamide 4.0 kg ai/ha post-emergence just before flowering	1354.5	33.2	32.3
6 Benomyl (seed dressing) 3 g/1 kg seeds	1193.1	74.3	74.2
7 Benomyl 0.5 kgai/ha at flowering	1302.6	82.6	80.0
8 Benomyl 0.75kgai/ha at flowering	1239.0	71.1	65.7
9 Diphenamid 2 kg ai/ha	891.6	6.3	7.1
10 Propyzamide 4.0 kg ai/ha when faba bean was 15 cm tall + glyphosate 0.08 kg ai/ha at flowering	1381.6	29.2	27.8
LSD at 5%	169.7	9.7	10.7

Diphenamid at the rate of 2 kg ai/ha significantly improved Orobanche control, but at the same time significantly decreased yield, due to a heavy phytotoxic effect on faba bean crop.

Yield was influenced by interaction between tillage systems and chemical application (Table 4).

Table 4. Effect of tillage systems and chemicals on yield of faba bean and Orobanche control.

Tillage System	Chemical Treatments	Yield kg/ha	No. Orobanche per m²	Total Dry Weight g/m²
Conventional (deep)	1	968.9	72.0	66.8
	2	1075.9	65.2	47.3
	3	1589.0	9.3	6.0
	4	1846.7	2.9	2.1
	5	920.9	39.9	34.2
	6	1046.1	67.4	64.6
	7	1041.9	64.7	59.4
	8	1165.2	64.5	56.1
	9	1022.9	3.2	4.6
	10	1050.0	29.3	27.8
Minimum Tillage (Shallow)	1	1433.2	89.6	109.7
	2	1491.4	56.6	48.6
	3	2363.6	12.5	9.6
	4	2306.0	6.6	6.8
	5	1430.9	32.9	35.3
	6	1162.7	83.8	85.1
	7	1394.5	93.7	91.2
	8	1422.5	79.9	80.7
	9	1176.1	8.9	80.7
	10	1546.5	38.0	33.0
Zero Tillage	1	1366.9	79.0	80.7
	2	1589.0	51.0	44.3
	3	2221.7	12.2	9.5
	4	2269.6	4.6	3.6
	5	1711.7	26.7	27.4
	6	1370.4	71.5	73.0
	7	1471.4	89.4	89.6
	8	1129.2	69.3	60.2
	9	475.9	7.0	6.7
	10	1548.2	22.1	22.5
LSD at 5% Tillage x Chemicals		293.9	17.1	18.6

The treatment with three glyphosate applications, significantly outyielded that with two applications of glyphosate on conventional tillage, while there was no significant difference in yield between these two chemical treatments on minimum and zero tillage. Orobanche control did not differ significantly between three and two applications of glyphosate on all investigated tillage systems.

Plots treated with propyzamide, at the rate 4.0 kg ai/ha when faba bean plants were 15 cm tall plus one application of glyphosate at flowering produced yield significantly higher with zero and minimum tillage compared to that with conventional tillage. Performance of these herbicides for Orobanche control (number and total dry weight) was more effective under zero tillage than under conventional and minimum tillage.

DISCUSSION AND CONCLUSIONS

The results of this investigation are at variance with those of other workers. A positive effect of deep tillage on Orobanche control was reported by Aleksiev (1967) and Saghir et al. (1978) while in our studies we found that minimum shallow tillage or zero tillage significantly increased the yield of faba bean. Further, Orobanche infestation was not significantly different between deep and zero tilled treatments.

Nitrogen was found to inhibit the parasite, which has also been indicated in previous studies (Kasasian, 1973, Saghir et al., 1978, Abu-Irmaileh, 1979), however the biochemical mechanisme of such effects is still unknown. The most effective measures of O. crenata control in this study appeared to be herbicide treatment especially glyphosate. Because it is translocated, the herbicide inhibits haustorial development at the beginning of its attachment to the roots of faba bean. The results confirm these in previous reports (Schmitt et al., 1979, ICARDA, 1979, 1981, 1982, Zahran, 1982).

Although the low dosage of the chemical required for effective control assumes low costs, the introduction of this control procedure on a large scale is difficult. Three applications of glyphosate at the beginning of faba bean flowering cause some operational problems and may thus discourage the farmers from adopting it. The effectiveness and the lower cost of two glyphosate applications integrated into minimum or zero tillage system found in our study indicate good prospects of this method for large-scale application. There is however, a need for further evaluation of this integrated control to verify the results. It is also worthwhile to evaluate the possibility of reducing the number of application further to one, by perhaps slightly increasing the dose of the product. These aspects will be evaluated during the 1983/84 season.

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THE SPECIES OF STRIGA IN EAST AFRICA

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Abstract. A dichotomous key to the 14 species of *Striga* in East Africa (Kenya, Uganda, Tanzania) is presented, together with references to taxonomic literature, synonyms and notes on hosts and geographical distribution.

INTRODUCTION

The following simplified key and summary notes have been prepared for the use of field workers, as well as for taxonomists with access to a botanical library and herbarium. A similar account with longer descriptions will be published in due course in the volume of the Flora of Tropical East Africa devoted to the Scrophulariaceae at present in preparation at Kew. (The abbreviated distribution details used here, e.g. U4, K3, refer to the regional divisions adopted by the Flora). In the meantime the present paper should enable workers to identify their material and to realise that there are large gaps in our knowledge of these plants. Although all the species are presumed to be obligate parasites, our ignorance of their host plants is evident for many of them. Careful field observations and experimental work on those species is urgently required. Further notes on habitat preferences and the range of habit and flower colour variation are also needed.

Attention may also be drawn to two other complementary accounts covering many of the same species that occur in East Africa. These are a forthcoming paper on the Sudanese species of *Striga*, by L.J. Musselman & F.N. Hepper in Kew Bulletin (1985), and the account of *Striga* by F.N. Hepper in Flora Zambesiaca (Vol. 8 1b Scrophulariaceae mainly by D. Philcox).

DICHOTOMOUS KEY TO THE EAST AFRICAN SPECIES OF STRIGA

1. Calyx 10– or more ribbed with at least 1 rib between each calyx tooth:
  2. Plant without hairs; leaves reduced to scales — 3. *baumannii*
  2. Plant hairy:
    3. Calyx 10-ribbed (i.e. with 1 extra rib to sinus between each tooth):
      4. Leaves 3–9 mm wide, coarsely toothed; flowers salmon-pink — 7. *forbesii*
      4. Leaves 1–2(–3) mm wide, more or less untoothed; flowers brilliant scarlet:
        5. Calyx-lobes triangular, acute but not subulate; plants usually well branched, slightly hairy — 8. *fulgens*
        5. Calyx-lobes narrow and acute; plants usually unbranched and harshly hairy:
          6. Flowers in opposite pairs, many out at the same time, corolla large — 5. *elegans*
          6. Flowers alternate, usually 2 or 3 out at the same time, corolla small — 1. *asiatica*
    3. Calyx 15– or more ribbed (i.e. with 2 extra ribs between each calyx tooth):
      7. Flowers salmon-pink; leaves 3–8 mm wide — 11. *latericea*
      7. Flowers white:
        8. Corolla-tube c. 20 mm long, 15–20 mm across — 14. *pubiflora*
        8. Corolla-tube 12–15 mm long, limb 10–12 mm across — 6. *euphrasioides*
1. Calyx 4–5 ribbed, each rib ending in a calyx tooth:
  9. Plants grey-hairy, the hairs appressed to the stems and directed downwards — 12. *linearifolia*
  9. Plants with erect or ascending hairs or glabrous:
    10. Stems glabrous or slightly pubescent, quite succulent — 9. *gesnerioides*
    10. Stems conspicuously or harshly pubescent:
      11. Corolla tube conspicuously hairy — 4. *bilabiata*
      11. Corolla tube almost hairless or with short hairs:
        12. Bracts linear to subulate:
          13. Bracts mostly shorter than the calyx — 2. *aspera*
          13. Bracts leafy, much longer than the calyx — 13. *passargei*
        12. Bracts lanceolate — 10. *hermonthica*

NAMES AND REFERENCES TO THE SPECIES IN EAST AFRICA

1. **Striga asiatica** *(L.) Kuntze*, Rev. Gen. Pl. 2: 466 (1891); Hepper in Fl. W. Trop. Afr. ed. 2, 2: 372 (1963); Hepper in Rhodora 76: 45 (1974); Agnew, Upland Kenya wild fl.: 565, fig. 568 (1974); Blundell, Wild fl. Kenya: 100 (1982).

Synonyms:
S. lutea Lour., Fl. Cochinch.: 22 (1790); Skan in Fl. Trop. Afr. 4(2): 409 (1906); Saldanha in Bull. Bot. Surv. India 5(1): 67–70 (1963); C.E. Smith in Rhodora 68(774): 167 (1966); Cramer in Rev. Handb. Fl. Ceylon 3: 400 (1981).
S. hirsuta Benth. in DC. Prodr. 10: 502 (1846).

A very variable slender herb, 5–30 cm high, simple or branched, often scabrid-hairy; flowers scarlet with yellow underside, occasionally yellow (especially in India).

Hosts: Cultivated maize and wild grasses; the commonest species of *Striga*.
Distribution: In East Africa in Uganda (U1–4), Kenya (K2–7) and Tanzania (T1–4, 6–8), throughout Africa and Asia, introduced into USA.

2. **Striga aspera** *(Willd.) Benth.* in Hooker's Comp. Bot. Mag. 1: 362 (1836); Skan in Fl. Trop. Afr. 4, 2: 403 (1906); Hepper in Fl. W. Trop. Afr. 2: 372 (1963).

Synonym:
Euphrasia aspera Willd., Sp. Pl. 3: 197 (1801).

Slender erect annual slightly hispid, 15–45 cm high, drying black; flowers pink or purple, sometimes white.

Hosts: Probably grasses.
Distribution: In East Africa known only from a few localities in Tanzania (T7, 8), elsewhere extending from Sudan to Senegal, becoming common in the west.

3. **Striga baumanii** *Engl.* Bot. Jahrb. 23: 515, t.12, figs. O–T (1897); Skan in Fl. Trop. Afr. 4, 2: 414 (1906); Agnew, Upland Kenya wild. fl.: 565, fig. p.568 (1974); Hepper in Fl. W. Trop. Afr. ed. 2, 2: 371 (1963); Agnew, Upland Kenya wild fl.: 565 (1974).

Glaucous leafless herb 60–80 cm. high; flowers dull yellow or maroon, in long slender spikes.

Hosts: Grasses in upland grassland.
Distribution: In E. Africa only known from Kenya (K3). Also occurs in Zaire, Cameroun and westwards to Sierra Leone and Mali.

4. **Striga bilabiata** *(Engl.) Kuntze*, Rev. Gen. Pl. 3, 2: 240 (1898); Hepper in Kew Bull. 16: 411 (1960).

Synonyms:
Buchnera bilabiata Thunb., Prodr. Fl. Cap.: 100 (1800).
Striga thunbergii Benth. in Hooker's Comp. Bot. Mag. 1: 363 (1836); Skan in Fl. Trop. Afr. 4, 2: 404 (1906).

S. bilabiata var. barteri *(Engl.) Hepper* l.c.: 414 (1960) & Fl. W. Trop. Afr. 2: 372 (1963).

Synonym:
S. barteri Engl. Bot. Jahrb. 23: 514, t.12 fig. E (1897); Skan in Fl. Trop. Afr. 4, 2: 406 (1906).

Perennial leafy herb 5–20 cm high, drying brown or greenish; flowers pinkish or violet.

Hosts: Grasses in swampy savanna that dries up and may be burnt.
Distribution: Near the East African Lakes in Uganda (U3) and Tanzania (T1), extending across West Africa to Senegal.

5. **Striga elegans** *Benth.* in Hooker's Comp. Bot. Mag. 1: 363 (1836); Skan in Fl. Trop. Afr. 4, 2: 408 (1906).

Erect perennial herb with slender usually unbranched pale green stems 15–35 cm high; flowers brilliant scarlet with yellow centre.

Hosts: Grasses in upland grassland.
Distribution: Only in East Africa; Kenya (K3, 6) and Tanzania (T1, 2, 4, 7).

6. **Striga euphrasioides** *Benth.* in Hooker's Comp. Bot. Mag. 1: 364 (1836); Skan in Fl. Trop. Afr. 4, 2: 412 (1906).

Erect annual 15–55 cm high, slender leafy stems usually slightly branched; flowers whitish in a long inflorescence.

Hosts: Probably grasses in sandy *Brachystegia* woodland.
Distribution: In East Africa only in southern Tanzania (T8); also in Malawi.

7. **Striga forbesii** *Benth.* in Hooker's Comp. Bot. Mag. 1: 364 (1836); Skan in Fl. Trop. Afr. 4, 2: 410 (1906); Hepper in Fl. W. Trop. Afr. ed. 2, 2: 371 (1963); Agnew, Upland Kenya wild fl.: 565, fig. p.568 (1974).

Erect, simple or little-branched rather scabrid herb, 10–60 cm high; flowers usually salmon-pink, occasionally scarlet or yellow.

Hosts: Setaria sphacelata.
Distribution: In E. Africa in Uganda (U1–4), Kenya (K3–7), Tanzania (T1, 3, 4, 6, 7), also widespread in tropical Africa and Madagascar.

8. **Striga fulgens** *(Engl.) Hepper* in Kew Bull. 38: 598 (1984).

Synonym:
Buchnera fulgens Engl. Bot. Jahrb. 28: 478 (1900); Skan in Fl. Trop. Afr. 4, 2: 392 (1906).

Erect perennial herb up to 50 cm high, with numerous slender branches; flowers bright scarlet or orange-red.

Hosts: Probably grasses in upland grassland.
Distribution: Only known from Tanzania (T7). A little known species collected only once at Uhehe, Tengulinyi, Iringa District by Goetze (No. 697) in 1899.

9. **Striga gesnerioides** *(Willd.) Vatke* in Oesterr. Bot. Zeitschr. 25: 11 (1875); Hepper in Fl. W. Trop. Afr. ed. 2, 2: 373 (1963); Agnew, Upland Kenya wild fl.: 565, fig. p.568 (1974).

Synonym:
S. orobanchoides Benth. in Hooker's Comp. Bot. Mag. 1: 361 (1836); Skan in Fl. Trop. Afr. 4(2): 402 (1906).

An erect perennial herb with usually rather stout tufted succulent stems a few cm high, or up to 30 cm, purple when fresh drying black, almost glabrous to rather pubescent; flowers purple to whitish.

Hosts: Reported as parasitic on tree roots, *Euphorbia*, and grasses including *Sorghum*, and cultivated *Brassica*.
Distribution: In East Africa in Uganda (U1–4), Kenya (K1–4, 6, 7), Tanzania (T1–4, 6–8), throughout tropical and South Africa, Arabia and India.

10. **Striga hermonthica** *(Del.) Benth.* in Hooker's Comp. Bot. Mag. 1: 365 (1836); Skan in Fl. Trop. Afr. 4, 2: 407 (1906); Hepper in Fl. W. Trop. Afr. 2: 372 (1963); Agnew, Upland Kenya wild fl.: 565 (1974).

Synonyms:
Euchnera hermonthica Del., Fl. Egypte: 245, t.34, fig. 3 (1813).
Striga senegalensis Benth. in DC. Prodr. 10: 502 (1846).

Erect leafy annual herb 25–40 cm high; flowers large and decorative pink in an elongated inflorescence.

Hosts: Cultivated *Sorghum, Pennisetum*, also wild *Brachiaria, Dactyloctenium* and other grasses.
Distribution: In East Africa in Uganda (U1–3), Kenya (K5), Tanzania (T1), and throughout the drier parts of tropical Africa and Madagascar.

11. **Striga latericea** *Vatke* in Linnaea 43: 311 (1882); Skan in Fl. Trop. Afr. 4, 2: 411 (1906); Agnew, Upland Kenya wild fl.: 565 (1974).

Erect annual simple stem or with a few branches, 15–50 cm high, scabrid-pubescent; flowers large, salmon pink.

Hosts: Grasses in thorn bush grassland.
Distribution: Only in Kenya (K1, 4, 7), Tanzania (T1, 3, 6, 8).

12. Striga linearifolia *(Schum. & Thonn.) Hepper* in Kew Bull. 14: 416 (1960) & in Fl. W. Trop. Afr. ed. 2, 2: 372 (1963); Agnew, Upland Kenya wild fl.: 565 (1974).

Synonyms:
Buchnera linearifolia Schum. & Thonn., Beskr. Pl. Guin.: 279 (1827); Hepper, W. Afr. Herb. Isert & Thonning: 119 (1976).
Striga canescens Engl., Pflanzenw. Ost-Afr. C: 361 (1895); Skan in Fl. Trop. Afr. 4, 2: 406 (1906); Hutchinson & Dalziel, Fl. W. Trop. Afr. ed. 1, 2: 227 (1931).
S. strictissima Skan in Fl. Trop. Afr. 4, 2: 407 (1906).

Erect annual herb, ± leafless stems simple or little-branched from near the base, ribbed and square, 30–60 cm high; flowers pale lilac or white in rather long dense spikes.

Hosts: Grasses in savanna grassland.
Distribution: In Uganda (U1–4), Kenya (K3, 4) and Tanzania (T1, 2, 4, 5, 7), throughout tropical Africa.

13. Striga passargei *Engl.* Bot. Jahrb. 23: 515, t.12, figs. M, N (1897); Skan in Fl. Trop. Afr. 4, 2: 403, note under *S. aspera*, (1906); Hepper in Fl. W. Trop. Afr. ed. 2, 2: 372 (1963).

Erect leafy slightly scabrid annual 10–45 cm high, stems simple or little branched, drying black; flowers creamy white to pale yellow with pink centre or pale purple.

Hosts: Probably grasses.
Distribution: In E. Africa only reported from Tanzania (T1, 4, 8); also in Sudan and westwards to Senegal.

14. Striga pubiflora *Klotzsch* in Peters, Reise Mossamb. Bot.: 227 (1861); Skan in Fl. Trop. Afr. 4, 2: 412 (1906).

Perennial with a tuft of erect stems, simple or branched above, 35–60 cm high; flowers large, white.

Hosts: Probably grasses in seasonally flooded savanna.
Distribution: In East Africa in Kenya (K7) and Tanzania (T3, 6, 8); also Malawi and Mozambique.