PD-AAW-711

UNITED STATES GOVERNMENT

memorandum

DATE: September 20, 1987

REPLY TO ATTN OF: George Ghobrial, AGR

SUBJECT: AID/SCI Proposal 5.382, Biochemical Aspects Unique to the Parasite (Striga)/Host (Sorghum) System. (Grant No. 936-5542-G-00-5027)

prg. #

TO: See Distribution

As requested in State Cable 088484 dated 24th March, 1985, a copy of a progress report for the period September 1, 1985 - September 30, 1986 on subject research project is enclosed.

Mission is considering a no cost extension for the project completion date to 31 October 1988, to enable principal investigator, Dr. S. Khalid, to reach final conclusions on this research and the date due for a final report to be adjusted accordingly.

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BIOCHEMICAL ASPECTS UNIQUE TO THE PARASITE (STRIGA)/HOST (SORGHUM) SYSTEM

Progress Report for the period

September 1, 1985 - September 30, 1986

Prepared for the

Agency for International Development

Under

Research Grant No. 936-5542-G-00-5027-00

Submitted by:

Dr. Sami A. Khalid
Principal Investigator

September 1986

Our research activities during the period September,1985 - December,1986 involved a number of agricultural and chemical aspects. Most notably, the collection of the plant material from two geographical locations (Shambat and Abu Naama), germination experiments and the phytochemical analysis of the collected specimens. Fresh plant material of shoots (aerial & subterranean) and seeds of *Striga hermonthica* strains were collected from Shambat (12 Km North of Khartoum) and Abu Naama (570 Km East from Khartoum) during September,85-May,86. The collected fresh plant material were separated into the following lots:

a) Fresh shoots (stored in 5% $CaSO_4$)

b) Fresh shoots (oven-dried at 80°C for 24 hr)

c) Fresh shoots (sun-dried for two weeks)

d) Seeds were threshed and cleaned.

Samples from each of the four lots were extracted with 70% methanol and then concentrated under vacuum using rotary evaporator. The remaining aqueous extract was consequently partioned with chloroform followed by ethyl acetate using Liquid/Liquid continous Soxhlet extraction technique. The reultant organic extracts and the residual aqueous phase were all concentrated *in vacuo* and chromatographed on silica gel G F_{254} thin layer chromatography (TLC) plates (developing system: ethyl acetate-methanol-water). The developed chromatograms were visualized/detected under ultra violet light and subsequently sprayed with vanilline/H_2SO_4 to locate the iridoids and any other secondary metabolites (e.g flavonoids). This experiment revealed the presence of four major flavonoids and two major iridoids in addition to some other fluorescent spots resemble in thir chromatographical behaviour compounds like the coumarins and cinnamic acid derivatives. The iridoids and flavonoids exhibited a high concentration in the ethyl acetate fraction in comparison with the chloroform and the aqueous fractions. The ethyl acetate fraction was subjected to further fractionation on polyamide column chromatography using water and water-methanol mixture as eluents.

The early eluted fractions (1-27) were collected and monitored by TLC using cellulose plates (n-butanol-acetic acid-water) while the rest of the fractions (28-77) were analyzed on silica gel G F254 plates (chloroform-methanol-water; lower phase) using the multiple development mode. Similar fractions were pooled, concentrated and further fractinated by counter-current-chromatography (solvent system: chloroform-methanol-water in different proportions). This work resulted in the isolation of two compounds resembling the flavonoid-glycosides in their chemical reactivity and two iridoids. The spectroscopical work in the structure elucidation of these compounds was carried out in the U.S.A by the principle investigator during his visit to the Old Dominion and Purdue Universities, in September 1986(see Trip Report). All the isolated compounds were subjected to further purification using High Performance Liquid Chromatography (HPLC) followed by recrystallization and spectroscopical investigation using the highly advanced Nuclear Magnetic Resonance Spectroscopy (NMR) . Although the structur of four compounds has been tentatively identified as two flavonoids and two iridoids, their final strctures still need some further advanced techniques to be fully resolved. The amount isolated was very small to obtain sufficient signal-to-noise to detect the enhancement of the different signals in the NMR spectrum in the Overhauser experiments. Therefore, we concentrate now on the isolation of a larger quantities from these compounds along with the other minor compoments of Striga.

The HPLC indicated a significant quantitative difference between Shambat and Abu Naama populations. Abu Naama population seems to accumolate more than double the amount of iridoids found in Shambat population. These results need further verification by using the new collection of this year with special statistical design and we shall use the Reversed Phase HPLC instead of the normal phase HPLC.

An special experiment was designed to compare the chemical components of two varities of sorghum. One is reputed to be susceptible (Dabr) and the other one is considered to be resistent (Framida) to Striga hermonthica infestation. Both Dabr and Framida were planted and the root exudate was obtained using suction pump. The root exudates were extracted with ethyl acetate, concentrated and injected into HPLC. A significant qualitative and quantitative differences were observed. These chemical differences may provide the only explanation available to explain the resistance and susceptibility of these two varities. To find out about the possible differences in the iridoid content and some other phenolic glycosides and their possible role in germination a different age-groups of seeds of Striga hermonthica (0-,1-,and 2-year old populations) were packed in vitex cloth and burried in plastic cups (6 cm in diameter) filled with 200g of sterlized sand and watered daily with distiiled water for 10 days. Seed exudates were collected using suction pump. The exudates were extracted with cold ethyl acetate and the ethyl acetate concentrate was subjected to counter-current-chromatography. The fractions collected from this experiment were screened by TLC. Sub-samples of the pre-conditioned seeds were subjected to a germination stimulant obtained from sorghum seedling grown in a plastic pots filled with sterlized sand. The results of the germination counts . were strikingly corresponding with the TLC pattern. This observation was specially true for Abu Naama population. However, the quantitative aspects of the leachets chemistry in relation to germination count will be carried out in the next stage of this project.

A set of experiments were designed to address the problem of translocation of the iridoids from the parasite to the host. Two sorghum varities were tested; one is reputed to be resistant (Framida) and the other susceptible (Dibaikri) to S. hermonthica infestation. We used the variety Dibaikri in this experiment due to the non availabilty of the Dabr variety.

Four seeds of sorghum cultivars were grown in clay pots (21 cm diameter) filled with 6 Kg soil. Mixture of river silt and sand in 3;1 ratio was used as potting soil. The soil mixture was sterlized by fumigation for 24 hours with methyl bromide. Striga infested treatment was obtained by addition of 0.1 g of Striga seed to the soil mixture. This involved the removal and placement of the top 3 cm of the soil from each pot in small tumbler kept rotating at an angle 45° for five minutes to ensure thorough mixing. Seed infested soil was returned to each pot and leveled out. Control treatment did not receive any striga seed . After the flowering of Striga the soil was washed from each pot and parasite and host were separated and extracted as described previuosly. No iridoids were detectable when we used normal TLC. However, High Perfromanc Thin Layer Chromatography (HPTLC) allowed the detection of trace amount of iridoids in the host.

Trip Report

I arrived in New York on 19 September and was met by my collaborator Prof. Lytton J. Musselman of the Old Dominion University. Through the courtesy of Prof. Musselman we were able to travel to the University of Virginia and to meet Dr. J. Riopel of the Department of Biology. Dr. Riopel has considerable experience on the physiology of Striga germination and development, and in the culture and management of Striga in the laboratory and greenhouse. He played a prominant role in the identification of the Striga haustorium initiation. His laboratory has long been one of the few in the US authorized to grow Striga. There we discussed some of our germination assays.

We returned to Nor Folk and we continued our discussion and interpretation of the results and I managed to present a seminar in the Old Dominion University. From Nor Folk we were able to travel to Raleigh and meet Prof. A. D. Worsham of the North Carolina State University. Prof. Worsham has a long standing contribution to Striga research and he was the first to establish the involvement of coumarins in the germination of Striga. On the 4 th of October I was able to travel to Indianapolis. I was met in Indianapolis by Prof. L. Butler of the Department of Biochemistry, Purdue University. After a long derive and through the courtesy of Prof. Butler we arrived in Purdue, West Lafayette in the late ours of Saturday. My arrival corresponded with the week-end holiday, but despite this I was able to spend the whole Sunday discussing research results, needs, and plans with Prof. Butler. Here in Purdue I was able to do all the chromatographical work with the HPLC which was allocated for me for more than a week. Dr. Butler was very kind in spending long hours with me in the lab. Dr. Butler has an extensive research program, including tissue culture, on the ployphenols produced by sorghum. It was in his laboratory that Dr. Netzly, postdoctoral researcher, discovered that sorghum root hair exude an oily

Material which was structurally identified, named sorgoleone, and found to be (in its reduced form) a potent germination stimulant for Striga, the first to be identified from a host plant.

While I was in Purdue we invited Prof. Riopel from Virginia and he was kind enough to come to Purdue to discuss further research possiblities to bring together a coordinated Striga research program and to maintain a strong collaborative effort linking the scientific personnel and expertise in both countries. I departed N.Y. on the 12th of October.

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Research Grant No. 936-5542-G-00-5027-00

Submitted by:

Dr. Sami A. Khalid
Principal Investigator

September 1986

PUBLICATION

Bebawi, F. F., Awad, A. E., Khalid, S. A., 1986. Germination, host reference and phenolic content of witchweed seeds. Weed Science 34(4) 529-532. (This work was accomplished prior to the actual award date of the grant but the work reported here includes an integral part of the proposed work).

PART A: COLLECTION OF MATERIAL

The research activities during this period concentrated on the collection of shoot and seed material of Striga from two geographical locations, Shambat and Abu Naama.

Fresh plant material of aerial and subterranean shoots of Striga hermonthica strains from Shambat and Abu Naama were collected and separated into three lots. The first lot, including fresh aerial and subterranean shoots, were stored in 5% $CaSO_4$ solution and sent to the phytochemistry lab. In addition, fresh aerial and subterranean shoot exudates were also sent to the phytochemistry lab.

The second lot of fresh aerial and subterranean shoot material of Striga was oven-dried at 80°C for 48 h. The oven-dry material was then ground in a plant mill and dispatched to the phytochemistry lab.

The third lot of aerial and subterranean shoot material of Striga was sun-dried for two weeks. The sun-dried material was then ground in a plant mill and sent to the phytochemistry lab.

Seed material from Shambat and Abu Naama plant populations were collected, threshed and cleaned. The seed of each Striga strain was then earmarked as 2-year old seed and stored in sealed glass jars under room conditions to be used in 1987.

PART B:

1. ISOLATION AND CHARACTERIZATION OF THE IRRIDOIDS OF TWO PHYSIOLOGICALLY DISTINCT POPULATIONS OF S. HERMONTHICA (Shambat and A. Naama).

Striga plant populations

	Shambat		Abu Naama	
	Aerial	Subterranean	Aerial	Subterranean
1	fresh intact material packed in 5% $CaSO_4$	fresh intact material packed in 5% $CaSO_4$	fresh intact material packed in 5% $CaSO_4$	fresh intact material packed in 5% $CaSO_4$
2	fresh plant extract obtained by plant hydraulic press	fresh plant extract obtained by plant hydraulic press	fresh plant extract obtained by plant hydraulic press	fresh plant extract obtained by plant hydraulic press
3	oven-dry material	oven-dry material	oven-dry material	oven-dry material
4	sun-dry material	sun-dry material	sun-dry material	sun-dry material

N.B. 16 Samples handed over to Dr. Khalid.

2. CHARACTERIZATION AND DETERMINATION OF IRRIDOIDS OF NON-PRECONDITIONED SEED

Striga seed populations

Shanbat		Abu Naama	
Seed Age	zero yr.	Seed Age	zero
	one yr.		one
	two yr.		two

Comments: Seed material was provided to Dr. Khalid to characterize and qualify irridoid content of fresh and after-ripened seed.

3. IRRIDOID CONTENT OF SEED PRECONDITIONED IN DISTILLED WATER

Striga seed populations

	Shambat	Results % Germination		Abu Naama	Results % Germination
Seed Age	zero yr.	2.1	Seed Age	zero	16.5
	one yr.	1.2		one	18.2
	two yr.	7.7		two	31.0
		3.7%			21.9%

Comments: Seed material was provided to Dr. Khalid to characterize the chemical constituent of the seed leachates and comelate leachate chemically with generation.

4. THE EFFECT OF N-FERTILIZER ON THE IRRIDOID CONTENT AND GERMINATION

Striga seed populations

physiological consequence on germination

Shambat

Urea (ppm)	Seed Age 0	1	2	Mean
0	2.1	1.2	7.7	3.7
5	2.8	8.1	15.3	8.7
10	0	0.5	0	0.1
20	0.4	1	0	0.5
40	2	8.5	2.6	4.4
80	0.5	0	0	.1
160	0	0.4	0	.1
320	0	0	1.1	.3
640	0	0	0	0
1000	0	0	0.3	.1
	.7	1.9	2.7	1.8

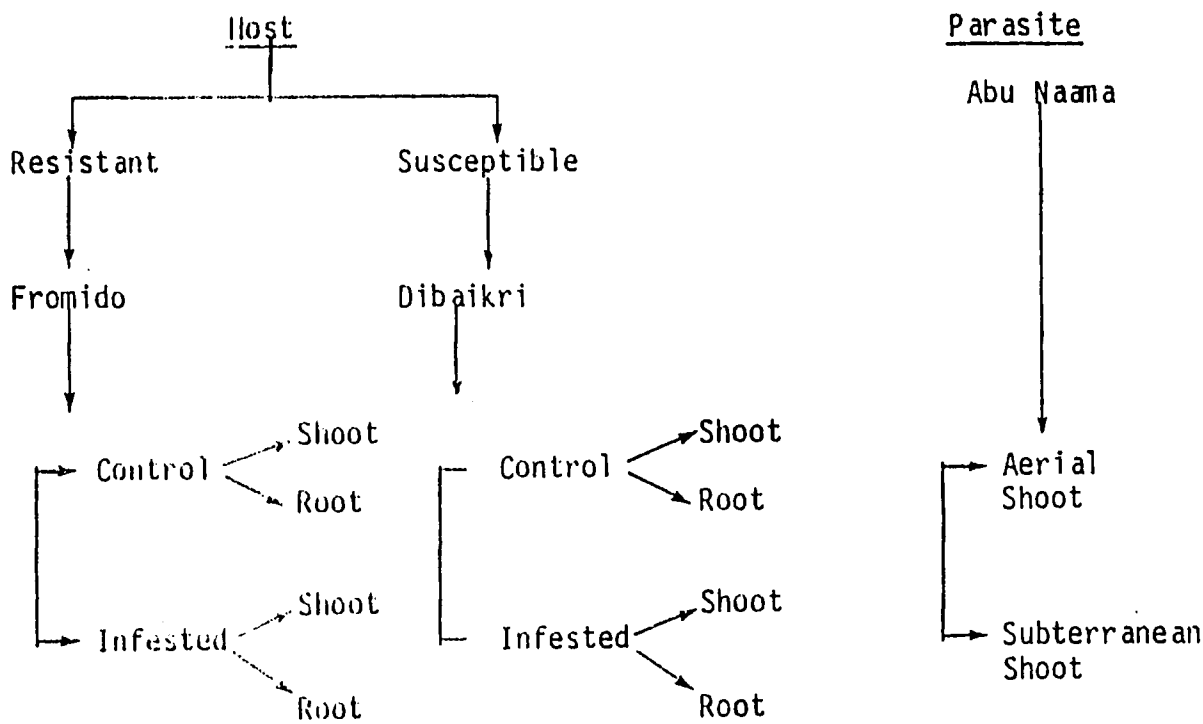
Abu Naama

Urea (ppm)	Seed Age 0	1	2	Mean
0	16.5	18.2	31.0	21.9
5	35.5	52.7	80.5	56.2
10	2.1	3	6.7	4
20	9.3	9.6	10.8	9.9
40	28.1	51.4	77.9	52.4
80	5.8	2.6	8	5.5
160	2.6	0	0.5	1
320	0	0	1.5	0.5
640	0	0	0.5	0.1
1000	0.9	0	0.3	0.4
	10.1	13.7	21.8	15.2

0	12.8%
5	32.5
10	2
20	5.2
40	28.4
80	2.8
160	.6
320	.4
640	0
1000	.2

*All urea concentrations other than 5 and 40 ppm have adversely affected seed germination.

5. TRANSLOCATION OF THE IRRIDOIDS FROM PARASITE TO HOST



* One part of the 10 samples has been packed in 5% $CaSO_4$. The other has been oven-dryed.

Weed Science. 1986. Volume 34:529–532

Germination, Host Preference, and Phenolic Content of Witchweed (*Striga bermonthica*) Seed Populations[1]

FAIZ F. BEBAWI, ADIL E. AWAD, and SAMI A. KHALID[2]

Abstract. Phenolic content, germination percentage, and host preference were compared among 11 seed populations of witchweed (*Striga bermonthica* (Del.) Benth. #[3] STRHE). Significant differences in germination percentage occurred among the seed populations after preconditioning by their exposure to different durations of warm-moist conditions. Greatest percentage of seed germinated after 8 days of preconditioning. Preconditioning for more than 16 days reduced percentage germination. The host specificity range of witchweed seed populations that parasitized pearl millet [*Pennisetum americanum* (L.) K. Schum] was broader than were those seed populations that attacked sorghum [*Sorghum bicolor* (L.) Moench.]. Results of thin-layer chromatography indicated large variations in phenolic content among the witchweed seed populations.

Additional index words. Germination inhibitors, STRHE.

INTRODUCTION

Intraspecific taxonomic differences have been used as a basis for identifying witchweed strains within species in Africa (4). Characters of diagnostic value in separating species include: number of ribs in the calyx, presence or absence of indumentum, relative sizes of calyx and corolla tubes, size of bract subtending each flower, and flower color.

Seed surface features and ecological characters have also been used to separate witchweed (*Striga*) species. The secondary ridges of the seed coat of *S. aspera* (Willd.) Benth. have prominent ornamentation with protuberances longer than those on the primary ridges as in some strains of *S. bermonthica* (10). *S. aspera* and *S. bermonthica* were separated based on their habitats. *S. aspera* was more commonly found in natural grassland, whereas *S. bermonthica* mainly occurs in cultivated sorghum fields[4].

It is uncertain whether taxonomic, ecological, and/or seed coat characters could form the bases for differences at the intraspecific level in witchweed. Only in *S. asiatica* (L.) Ktze. #[3] STRLU of differing geographical origin was it possible to discern differences in seed surface features. In general, seed from Asia have a more highly ornamented seed coat. Conversely, various geographical strains of *S. gesneroides* (Willd.) Vatke and *S. bermonthica* were indistinguishable on the bases of seed coat characters (10).

Before witchweed seed can germinate they must be subjected to: a) an afterripening period, b) a preconditioning period where seed are exposed to warm, moist conditions, and c) postconditioning period where preconditioned seed are exposed to a chemical stimulant exuded from the roots of host or some false-host plant (9).

Differences in seed germination and host parasitization within witchweed species have recently led to the identification of strains of both *S. bermonthica* and *S. asiatica*[5,6] (1, 7, 13). While species such as *S. bermonthica* can attack both sorghum and pearl millet, there are strains within the species that will attack mainly sorghum and others that attack mainly pearl millet (13). Differences in germination behavior have been attributed to the development of host specificity between and within species, which has resulted in species or strains responding to different germination stimulants (12).

Recent studies in Sudan indicate the presence of physiological variants (strains) of witchweed (1). Apparently, the progress in selection of cereal crops resistant to witchweed is delayed because there are physiological strains of the weed. Therefore, the identification and characterization of witchweed strains are essential elements in a research program, the aim of which is to identify and develop cereal crop varieties with broad-spectrum stable resistance to witchweed. The present study was conducted to compare the phenolic content of witchweed seed populations in relation to germination and host specificity of witchweed.

MATERIALS AND METHODS

Three-yr-old seed of 11 witchweed populations were collected from plants parasitizing sorghum and pearl millet from different geographical regions of the Sudan (Table 1). Seed were stored in unsealed glass jars under room conditions. Viability was determined for all populations by placing 30 to 35 seed in plastic petri dishes (60 by 15 mm) on 8-mm glass fiber filter paper moistened with 9×10^{-2} M triphenyl tetrazolium chloride solution. The petri dishes were stored in the dark for 8 days at 33 C. Seed viability range was no less than 96% for all witchweed seed populations.

[1] Received for publication July 23, 1985, and in revised form October 10, 1985.

[2] Assoc. Prof. and Res. Asst., Faculty Agric., Univ. Khartoum, Shambat, Sudan; and Lect., Faculty Pharm., Univ. Khartoum, P.O. Box 1966, Khartoum, Sudan.

[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4] Musselman, L. J. and C. Parker. 1983. Biosystematic research in the Genus Striga (Scrophulariaceae). Pages 19–24 *in* Proc. 2d Int. Workshop on Striga. Available from ICRISAT, Pantancheru, A.P. India.

[5] Lakshmi, B. and R. Jayachandra. 1979. Physiological variations in *Striga asiatica*. Pages 132–143 *in* Proc. 2d Int. Symp. Parasitic Weeds. Crop Sci. Dep., North Carolina State Univ., Raleigh, NC 27650, U.S.A.

[6] Parker, C. and D. C. Reid. 1979. Host-specificity in Striga species. Some preliminary observations. Pages 79–90 *in* Proc. 2d Int. Symp. Parasitic Weeds. Crop Sci. Dep., North Carolina State Univ., Raleigh, NC 27650, U.S.A.

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Table 1. Geographical locations and host plant from which witchweed seed populations were collected for seed germination and phenolic content determinations.

Population	Location (latitude, longitude)	Host
Gabob	14°20′ N, 35°40′ E	Sorghum
Rabk	10°19′ N, 32°25′ E	Sorghum
Renk	11°45′ N, 32°25′ E	Sorghum
Abu Naama	12°44′ N, 34°09′ E	Sorghum
Medani	14°23′ N, 33°29′ E	Sorghum
Maridi	4°55′ N, 29°28′ E	Sorghum
Shambat	15°40′ N, 32°21′ E	Sorghum
Kazgail	12°05′ N, 30°10′ E	Sorghum
Obeid	13°10′ N, 30°14′ E	Sorghum
Kazgail (pearl millet)	12°05′ N, 30°10′ E	Pearl millet
Obeid (pearl millet)	13°10′ N, 30°14′ E	Pearl millet

A factorial experiment was used to determine the germination percentage of the witchweed seed populations. It included six preconditioning treatments (exposure of witchweed seed to warm (33 C), moist conditions for 1, 2, 4, 8, 16, or 32 days) and 11 witchweed seed populations. There were 15 replications for each treatment, and the experiment was conducted twice.

Seed of both sorghum var. 'Dabar 1' and witchweed were initially sterilized by soaking them in a 1% sodium hypochlorite solution for 5 min followed by thorough washing with distilled water. The witchweed seed were then placed on 8-mm discs of moist glass fiber filter paper as described by Parker et al.[7]. There were approximately 30 to 35 witchweed seed per disc, and the discs were placed in a dark incubator at 33 C.

Meanwhile, 15 sorghum seedlings were grown from pregerminated seed in plastic cups (6 cm diam) filled with 250 g sterilized sand and watered with 30 ml distilled water for the production of root exudates. Four cups were sown with sorghum, which was left to grow for 10 days before root exudates were extracted. The cups with sorghum seed were placed in a glasshouse maintained at 30 to 35 C.

In the postconditioning treatment, discs of preconditioned witchweed seed were dried on filter paper to remove excess moisture and placed in a petri dish which contained glass fiber filter paper moistened with the sorghum root exudates. The dishes were closed, wrapped in polyethylene bags, and incubated at 33 C for 24 h. Germinated witchweed seed, as indicated by the emergence of the radicle, were then counted. Analysis of variance was performed on actual germination values and means separated by Duncan's multiple range test ($P \leq 0.05$).

Differences in witchweed host parasitization were investigated using split-plot experimental design in the glasshouse. Two witchweed susceptible crops, sorghum var. Dabar 1 and pearl millet var. 'Baladi', served as the main plot effect and the 11 witchweed seed populations represented the subplots. There were three replications and the experiment was conducted twice.

A potting mixture of river silt and sand (3:1, v/v) was sterilized at 160 C for 24 h before addition of witchweed seed. Witchweed infestation treatments were created by thoroughly mixing 1 mg of witchweed seed with 5 kg soil and then filling clay pots (21 cm diam) with the mixture. Control treatments did not contain witchweed seed. Four seed of sorghum and eight seed of pearl millet were planted 1 cm deep in separate pots and then thinned to one plant per pot. The experiment lasted for 12 weeks. Parasitization was determined by washing the host root under slow-flowing tapwater to remove all soil particles and expose the attached witchweed plants. The number of witchweed plants attached to the host roots was counted. Analysis of variance was performed on the number of attached witchweed plants.

Total phenolic contents of nonpreconditioned witchweed seed populations were determined except the "Kazgail' sorghum and pearl millet populations, which were not included because of insufficient supply of seed. Seed (5 g) were defatted with petroleum ether and then extracted with 70% methanol at 60 to 80 C in Soxhlet apparatus. The extracts were filtered and the filtrates concentrated in vacuo. The resultant aqueous extracts were then separately partitioned with ethyl acetate. The ethyl acetate fractions were concentrated in vacuo and chromatographed on precoated silica-gel G-plates[8] (0.2 mm) using toluene:ethylacetate:formic acid (5:4:1, v/v/v) as the developing solvent system. The chromatograms were visualized in daylight, under UV light and by reaction with 2% ethanolic $FeCl_3$ and vanillin (1%) in concentrated H_2SO_4, followed by heating the chromatographic plates at 110 C for 5 min. Glycosides were hydrolyzed by 2N HCl in boiling water followed by dilution with water and extraction with ethyl acetate.

RESULTS AND DISCUSSION

These studies suggest that large intraspecific variation exists among witchweed seed. Significant differences ($P < 0.01$) in seed germination percentages and numbers of attached witchweed plants occurred among the seed treatments (Table 2 and 3). Maximum seed germination and number of attached witchweed plants occurred with the 'Gabob' seed population. This finding is in agreement with that of Bebawi (1). Lowest germination percentage occurred with the Kazgail and 'Obeid' millet seed populations (Table 2), but these populations were able to attack sorghum (Table 3). Bebawi (1) also reported that the pearl millet seed population of witchweed were germinated in the presence of sorghum root exudates. The relatively low germination percentage of the pearl millet seed population is consistent with other reports[4].

[7] Parker, C., A. M. Hitchcock, and K. V. Ramiah. 1977. The germination of Striga species by crop root exudates. Techniques for selecting resistant crop cultivars. Pages 67–74 *in* Proc. Asian-Pac. Weed Sci. Soc. Conf.

[8] E. Merck, Schuchardt, 8011 Hohenbrunn bei Munchen, West Germany.

Table 2. Germination of witchweed seed from selected populations as affected by length of preconditioning treatment.

Population	Germination after preconditioning (days)[a]					
	1	2	4	8	16	32
	(%)					
Gabob	67 j	36 g	23 ef	92 l	42 h	14 d
Rabk	34 g	17 d	18 de	87 l	25 f	0 a
Renk	14 d	13 cd	7 b	78 k	42 h	4 a
Abu Naama	11 cd	22 e	16 d	73 k	26 f	5 a
Medani	15 d	14 d	9 bc	91 l	15 d	2 a
Maridi	0 a	3 a	4 a	76 k	10 bc	4 a
Shambat	3 a	10 c	2 a	53 i	6 b	1 a
Kazgail	1 a	2 a	2 a	47 h	11 bc	2 a
Obeid	0 a	0 a	0 a	18 de	2 a	1 a
Kazgail	0 a	0 a	0 a	9 bc	1 a	0 a
Obeid	0 a	0 a	0 a	8 b	2 a	0 a

[a]Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Germination of seed from all witchweed populations was significantly ($P < 0.01$) affected by the preconditioning treatments (Table 2). Maximum witchweed seed germination occurred at 8 days after preconditioning. With a few exceptions, the lowest seed germination percentage occurred at 32 days after preconditioning. The reduction of witchweed seed germination as a consequence of prolonged preconditioning was described as 'wet dormancy' by Vallance (11). Wet dormancy observed in our study is consistent with that reported by Reid and Parker[9] and Vallance (11). Wet dormancy may be of survival value to witchweed because witchweed seed in a state of wet dormancy are unable to respond to germination stimulants (11). Hence, chemical control of witchweed by synthetic germination stimulants such as ethylene gas (3, 6) and cultural control by both 'trap' and 'catch' crops becomes ineffective when witchweed is in a state of wet dormancy. In addition, in rainfed areas of Sudan where sorghum is planted late in the season, e.g., in August or September, when the soil remains continuously moist, wet dormancy of witchweed seed is likely to occur. Thus, sorghum sown late in the season is likely to escape severe witchweed infestations.

The interaction of preconditioning with seed population was also significant (Table 2). This suggests that the seed germination of the seed populations was differentially affected by the preconditioning treatment. For example, germination of the Gabob seed population was greater after 1 than 2 days of preconditioning, while that of Shambat was greater after 2 than 1 day of preconditioning. The Gabob population, in comparison with the other seed populations, responded with relatively high seed germination (68%) within the shortest possible preconditioning time (Table 2).

Kazgail and Obeid pearl millet witchweed seed populations parasitized the sorghum host, whereas the sorghum witchweed seed populations were unable to parasitize the pearl millet host (Table 3). This suggests that the witchweed sorghum populations have a narrower host specificity than do witchweed pearl millet populations.

The Gabob population was the most distinctive of the subject groups relative to chromatographic pattern (Table 4). Witchweed populations from Medani, Abu Naama, Renk, and Rabk produced similar chromatographic patterns. 'Maridi', 'Shambat', and Obeid (sorghum and pearl millet, respectively) populations contained one spot with R_f value of 0.51, while Maridi population had an extra immobile spot. All the compounds were dark absorbing under UV light and showed green to black colors with $FeCl_3$ and yellow colors with vanillin/H_2SO_4 in the daylight. Hydrolysis of the extracts with 2N HCl and their subsequent extraction with ethyl acetate indicated their glycosidic nature. These abstracts produced compounds (aglycones) similar to those in the original extracts. A major spot ($R_f = 0.64$) and a trace amount of the parent immobile glycoside were present in the Gabob population.

Water-soluble phenolic compounds that contribute to dormancy are leached out of the seed (2, 5, 8). Therefore, the function of the preconditioning treatment is primarily to leach out these inhibitors. Chromatography of the ethyl acetate fractions of witchweed seed populations indicated variations in phenolic contents (Table 4). We envision that the germination inhibitor is a highly polar phenolic compound(s) which may be a phenolic glycoside(s) ($R_f = 0$) of flavonoidal nature.

Differences in phenolic compounds within seed populations of witchweed may explain the variations observed in germination responsiveness and host specificity of witch-

Table 3. Mean number of attached witchweed plants from seeds of different populations on sorghum[a].

Population	Sorghum
	(no.)
Gabob	51 e
Rabk	42 d
Renk	29 bc
Abu Naama	38 cd
Medani	44 de
Maridi	23 b
Shambat	16 ab
Kazgail	24 b
Obeid	36 cd
Kazgail	12 a
Obeid	9 a

[a]Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

[9]Reid, D. C. and C. Parker. 1979. Germination requirements in Striga species. Pages 202–210 *in* Proc. 2d Int. Symp. Parasitic Weeds. Crop Sci. Dep., North Carolina State Univ., Raleigh, NC 27650.

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Table 4. R_f values of phenolic compounds in witchweed seed populations from differing geographical locations in the Sudan.

Witchweed seed population	R_f
Gabob	0.64, 0
Abu Naama	0.63, 0.51, 0
Medani	0.63, 0.51, 0
Rabk	0.63, 0.51
Renk	0.63, 0.51
Maridi	0.51, 0
Shambat	0.51
Obeid	0.51
Obeid	0.51

weed. The seed populations studied may be arbitrarily divided into three distinct groups. One group is characterized by high R_f values and germination percentage and is represented by the Gabob strain. The second group is characterized by intermediate R_f values and germination responsiveness and is represented by the 'Abu Naama', 'Medani', 'Rabk', and 'Renk' strains. Obeid (sorghum and pearl-millet respectively), Kazgail (sorghum and pearl millet respectively), Shambat, and Maridi represent the third group which is characterized by low R_f values and germination percentages. Our data also indicate that host specificity is more highly developed within the sorghum than the millet strains of witchweed.

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