Effects of 2,4-D , DMBQ and sorghum root extract on haustorium induction and attachment of witchweed [*Striga hermonthica* (Del.) Benth.] to roots of *Sorghum bicolor* (L.) Moench

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ABSTRACT

Two types of experiments were conducted during 2006 in Wad Medani, Sudan in the laboratory. *In vitro* experiments were carried out to study the effects of 2,4-D at 2.5-100 M, 2,6-dimethoxy-ρ-benzoquinone at 2.5 and 10.0 M and sorghum root extract at 6.3 and 25 g/L on haustorium induction of witchweed. *In vivo* experiments were carried out to study the effects of 2,4-D at 2,5-100 M for pre-conditioning and soaking pre-conditioned witchweed seeds at the same rate for 10-120 min on attachment of haustorium to sorghum seedling roots. Treatments were arranged in a completely randomized design with three replicates. Witchweed germilings were examined for haustorium induction and attachment 4 and 15 days after treatment. Data were collected, transformed to arcsine as required and subjected to the analysis of variance procedure. The results revealed that haustorium induction and attachment to sorghum seedling roots was suppressed with increased 2,4-D concentration(2.5-100 M) and soaking period (10-120 min) of pre-conditioned seeds. However, haustorium induction increased with increased 2,6-dimethoxy-ρ-benzoquinon (2.5 and10.0 M) and sorghum root extract concentration (6.3 and 25g/L). Much remains to be learnt about the action of 2,4-D. It appears to have a potential for Witchweed management. However, further research is needed to clear the basic principles of 2,4-D actions on Witchweed.

INTRODUCTION

Witchweed [*Striga hermonthica* (Del.) Benth.] (Orobanchaceae) is the most damaging species of *Striga*. It is native to Africa and does major damage to sorghum [*Sorghum bicolor* (L.) Moench] and millet [*Pennisetum glaucum* (L.) R. Br.]. The adverse effects of witchweed on grain yield of sorghum vary from 70% to 90% on improved culture and 40% to 50% on traditional culture (Showemimo, 2003; Showemimo and Kimbeg 2005; Showemimo, 2006). The parasite produces a large number of small seeds, which are dormant and require a conditioning period in a warm moist

environment before they have the potential to germinate (Yukihiro *et. al.,* 2006). Germination occurs in response to host-derived signal molecules, collectively named strigolactones.

Once the seeds of witchweed have germinated up to several mms from a host root, they have to make contact with that root in order to parasitize it (Dixon and Parker, 1984). On contact with a host root, the elongation of the radicle stops and the development of the haustorium immediately begins. This process has been shown to depend on a haustorium-imitating substance. The substance responsible for initiating haustorial development in *S. asiatica* has been identified as 2,6-dimethoxy-ρ-benzoquinone (2,6-DMBQ) (Smith *et al.,*1990).Lynn and Chang (1990) revealed that 2,6-DMBQ cannot normally be detected in the exudates from sorghum roots, although it is present in its extract. Many phenolic and flavonoid substances can also initiate haustoria development in both *S. asiatica* and *S. hermonthica* (Chang and Lynn, 1986), presumably acting as the substrate for the production of 2,6-DMBQ *via* on enzyme system. Cytokinins are also known to be initiators of haustorial development. Thidiazuron, which has some cytokinin activity, can also initiate haustorial development (Babiker *et al.,*1992).Haustoria-like structures and the early stages of nodule development (Crespi and Galvez, 2000) are induced by cytokinins, whereas indole acetic acid appears to be important for initiation, morphogensis and continued development of lateral roots (Deklerk *et al.,*1999). Compounds that inhibit auxin transport also induce nodule-like structures (Hirsch *et al.,*1989). In *S. asiatica*, the radicles are most responsive and more able to form haustoria within 4 days of germination (Parker and Riches, 1993).

The process of haustorial development and penetration of the host is similar in *S. hermonthica* (Olivier *et al.,* 1991) and *S. asiatica* (Ramaiah *et al.,* 1991). Sticky hairs on the young haustorium help the parasite germiling to adhere to any surface. After attachment by these hairs, intrusive cells develop at the root tip and penetrate the cortex of the host. Because of its parasitic nature, witchweed interaction with its host plays a crucial role in its survival. Throughout the life cycle of witchweed, there are several sequential host-parasite interactions and their disruption offers unique opportunities for controlling this harmful parasite (Ejeta *et al.,*1992).Therefore, the present investigation was conducted to study the effects of 2,4-D, 2,6-dimethoxy-ρ-benzyoquinone (DMBQ) and sorghum root extract (SRE) on haustorium development and attachment to sorghum roots.

MATERIALS AND METHODS

The disc technique described by Dafaallah (2006) was used in this study. For pre-conditioning of *Striga* seeds, about 80-100, glass fiber filter paper (GFFP) (Whatman GF/C), discs (0.5 mm diameter) were placed on one layer GFFP in 9 cm glass Petri dish (GPD). Witchweed seeds (25-100) were sprinkled on each disc. The seeds were moistened with 4.5 ml distilled sterilized water, sealed with para film, covered with black polyethylene bag and incubated at 30°C in the dark for 12 days.

GR 24 preparation

GR 24 (1 mg) was dissolved in 1 ml acetone. Sterilized-distilled water was added to give a volume of 10 ml resulting in stock solution of 0.1 g/L (100 ppm). GR 24 concentration was prepared by sequential dilution of the stock solution with sterilized-distilled water to give 0.1

ppm solution. Discs containing conditioned *Striga* seeds were placed on top of similar glass fiber filter paper discs in a Petri dish. GR 24 at 0.1 ppm was applied to each pair of discs. The Petri dishes were sealed with Para film, placed in black polyethylene bags and incubated at 30°C in the dark for 2 days for germination.

***In vitro* experiments**

In vitro experiments were designed to study the effects of 2,4-D at 2.5-100 □M, 2,6-dimethoxy-ρ-benzoquinone and sorghum root extract on haustorium induction of witchweed. 2,6-dimethoxy-ρ-benzoquinone (DMBQ) (1.77 mg) was dissolved in 1ml sterilized-distilled water. Sterilized-distilled water was added to give a volume of 10 ml resulting in stock solution of 0.177 mg/L (1 □M). The test solutions (DMBQ) at 2.5 to100□M were prepared by dilution of the stock solution with sterilized-distilled water. Sorghum root extracts (SRE) were prepared by maceration of 1 g of freshly harvested roots in 10 ml water resulting in a stock solution of 100 g/L. Various dilutions of (SRE) at 3.1-50.0g/L were prepared from the stock solution with sterilized-distilled water. The test solutions of (DMBQ) at 2.5 to 100□M or (SRE) at 3.1-150.0 g/L were added at 40 □L to each disc, placed in a plate, containing 2 days *Striga* germilings. The plates were sealed with para film, covered with black polyethylene and incubated at 30°C in the dark for 2 days old. *Striga* germilings were examined for haustorium induction 2 days after treatments with DMBQ or SRE. Concentrations that displayed the lowest and highest haustorium induction were selected for further experimentation. The selected test solutions, DMBQ at 2.5 and 10□M and SRE at 6.3 and 25g/L mixed with 2,4-D at 2.5, 5, 10, 20, 40, 80 and 100□M in a total volume of 40□L and added to each disc, placed in a plate containing 2 days old *Striga* germilings. Untreated control (without 2,4-D) was included for comparison. *Striga* germilings which were induced by GR24, as previously described, were used. The plates were sealed, covered with black polyethylene and incubated at 30°C in the dark for 2 days. *Striga* germilings were then examined for haustorium formation as previously described.

***In vivo* experiments**

Sorghum seedlings (15 days old) were placed on glass fiber filter papers on rock wool inside plastic dishes (9 cm i.d), with a lateral opening to allow for emergence of sorghum shoots. *Striga* seeds, conditioned in 2,4-D at 2.5-100 □M for 7 and 12 days, seeds conditioned in water and then treated with 2,4-D at 20 and 80 □M for 10, 15, 30, 60 and 120 min or left untreated (without 2,4-D) were transferred and placed near the roots of sorghum seedlings. Sterilized-distilled water was added to each Petri-dish as needed.

The Petri-dishes were sealed, placed in black polyethylene bags and incubated at 30±0.5°C in continuous light. Treatments were arranged in a completely randomized design with three replicates. *Striga* germilings were examined for haustorium induction and attachment 15 days later. Data were collected, transformed to arcsine as required and subjected to the analysis of

variance procedure. Where the test was significant, separation of means was done using Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

***In vitro* experiments**

Effects of 2,4-D on haustorium induction by 2,6-dimethoxy-ρ-benzoquinone (DMBQ)

DMBQ at 2.5 and 10 □M induced 29% and 90% of four–days-old *Striga* germilings to produce haustoria, respectively (Fig. 1). In *S. asiatica*, the radicles were most responsive and best able to form haustoria within 4 days of germination and at 30°C may begin to lose this ability even 2 days after germination (Parker and Riches, 1993). Previous findings by Chang and Lynn (1986) showed that 2,6-DMBQ is responsible for initiating haustorial development in *S. asiatica*. Keyes *et al.*(2000) reported that when two-days old. *S. asiatica* seedlings were incubated at 30°C in 1 ml of H_2O containing 10 □M DMBQ, the seedlings showed typical swollen root tips with haustorial hair formation after 24 h. In the terminal haustoria formed in *S. asiatica*, organogenesis was manifested primarily in the redirection of cellular swelling events. The cells distal to the meristem switch from longitudinal to radial growth, and the circumscribed pre-epidermal cells form haustorial hairs. The extent of swelling required for the new organ could be significant, with an increase in diameter from twofold to fourfold, and development was complete within 24 h of induction. The swollen cells created a larger surface area likely to be critical for host attachment, and it was within this swollen tip that the haustorial primordial form, giving rise to the infection peg and ultimately the mature host interface (Riopel and Timko, 1995).

2,4-D added in mixtures with DMBQ reduced haustorium formation. The inhibitory effects of 2,4-D progressively increased with concentration. 2,4-D at 2.5 and 5□M depressed haustorium induction by DMBQ, *albeit* not significantly different when compared to the corresponding DMBQ concentration without 2,4-D. Increasing 2,4-D concentration to 10□M or more resulted in a significant inhibition of haustorium induction. Inhibition of haustorium induction was greater than 80% at 2,4-D concentration of 40-100□M. 2,4-D was described as a plant growth regulator and an efficient herbicide. The chemical appeared to concentrate on young embryonic or meristemic tissues that wrer growing rapidly (Klingman *et al.*, 1982). It had occasionally prevented witchweed attack and reduced crop damage (Langston and English, 1990). Haustoria-like structures and the early stages of nodule development (Crespi and Galvez 2000) were induced by cytokinins, whereas IAA appeared to be important for initiating, morphogenesis, and continued viability of lateral roots (Deklerk and *et al.* 1999). Compounds that inhibited auxin transport (Hirsch and *et al.* 1989) also induced nodule-like structures. Keyes *et al.* (2000) observed that auxins were very potent inhibitors of haustorium induction when two-days-old *S asiatica* seedlings were incubated at 30°C in 1 ml of H_2O containing either kinetin or 10 □M DMBQ with α-IAA.

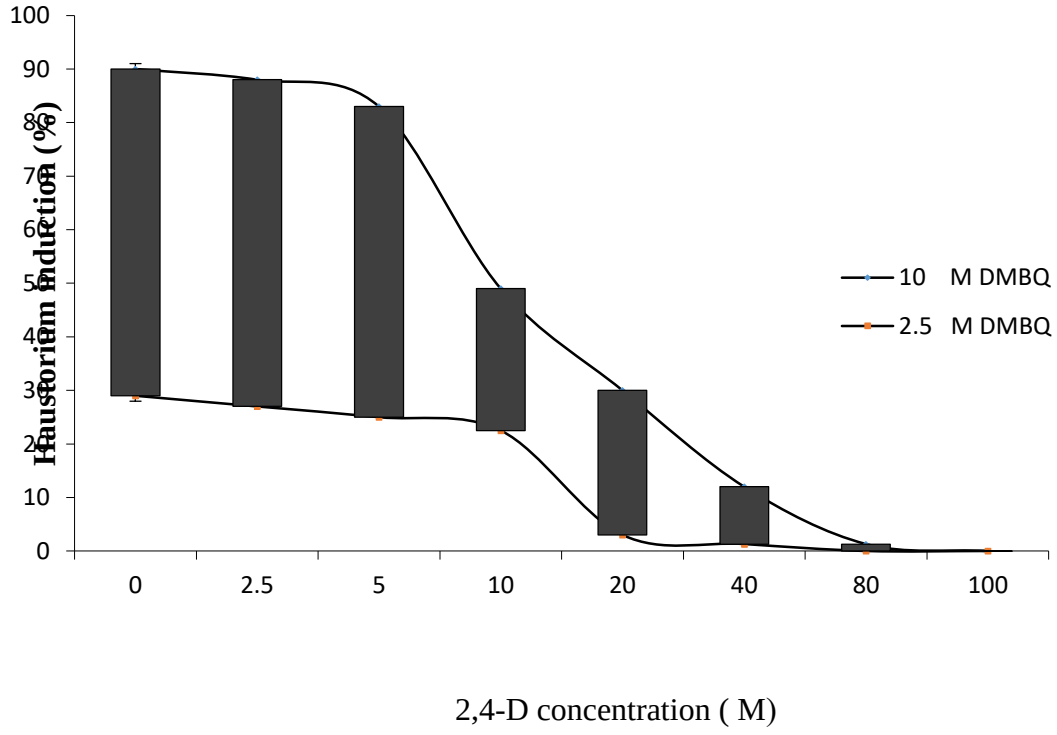

Fig. 1. Effects of 2,4-D and DMBQ on haustorium induction in witchweed germiling.

Effects of 2,4-D on haustorium induction by sorghum root extract (SRE)

Sorghum root extract at 6.3 and 25g/L induced 24% and 72% of four–days-old *Striga* germilings to produce haustoria, respectively (Fig. 2). Lynn and Chang (1990) revealed that 2,6-DMBQ could not be normally detected in the exudates from sorghum roots although it was present in extracts of sorghum root. 2,4-D inhibited haustorium induction in response to the root extract. The inhibitory effects of 2,4-D progressively increased with concentration. 2,4-D at 2.5□M or more significantly inhibited haustorium induction. For the diluted extract, haustorium induction was completely inhibited by 2,4-D at 10-20 □M. However, for the concentrated extract, complete inhibition of haustorium induction was attained at a concentration of 40□M. Haustorium initiation involved cell division and differentiation (Riopel *et al.* 1990). 2,4-D was known to be inhibit differentiation of cell and led to callus formation.

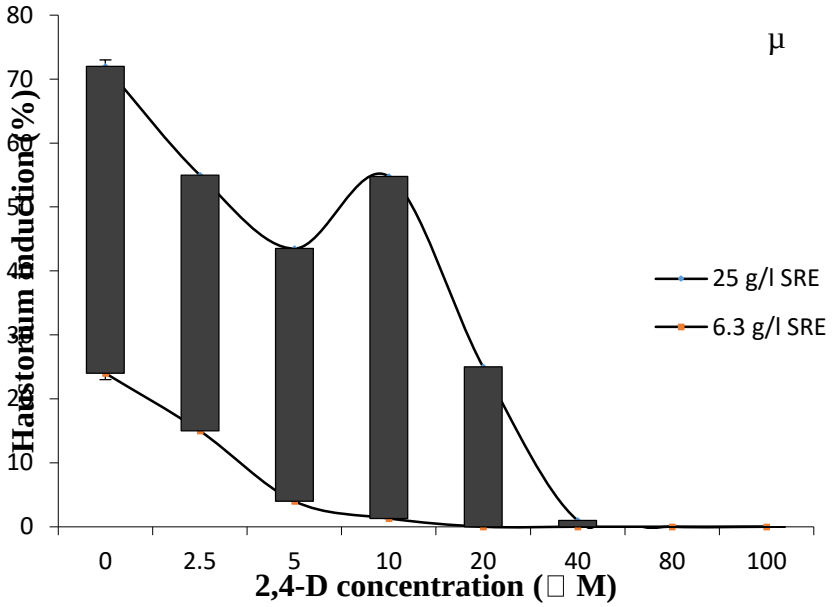

Fig. 1. Effects of 2,4-D and DMBQ on haustorium induction in witchweed germiling.

***In vivo* experiments**

Effects of pre-conditioned *Striga* seeds in 2,4-D for 7 days on haustorium attachment to sorghum roots

Successful attachment (92.7%) of *Striga* germilings to sorghum roots was achieved when seeds were conditioned in water for 7 days (Table 1). However, for the seeds conditioned in 2,4-D at 20 and 80□M for 7 days, attachment of *Striga* germilings to sorghum roots was only 23.3% and 4.3%, respectively.

Table 1. Effects of pre-conditioning witchweed seeds in 2,4-D for 7 and 12 days on haustorium attachment to sorghum roots

Haustorium attachment (%)		2,4-D concentration
Period (12 days)	Period (7 days)	(M)
95.3 a	92.7 (74.8) a	0
66.3 b		2.5
57.7 c		5
43.0 d		10
29.0 e	23.3 (29.0) b	20
12.3 f		40
5.0 g	4.3 (12.1) c	80
3.0 g		100
0.80	1.97	S.E±
3.55	4.8	CV (%)

* Data between parentheses are arcsine transformed.
* Means in same column followed by different letter(s) are significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

Effects of pre-conditioned *Striga* seeds in 2,4-D for 12 days on haustorium attachment to sorghum roots

Conditioning *Striga* seeds in 2,4-D at 2.5□M for 12 days significantly reduced haustorium attachment by 66.33 (Table 1). The inhibitory effects of 2,4-D progressively increased with concentration. Inhibition of haustorium attachment was greatest (5 and 3) at 80 and 100□M 2,4-D concentration, respectively. These findings are consistent with previous reports that conditioning of *S. hermonthica* seeds in 2,4-D suppressed germination irrespective of stimulant concentration (Dafaallah, 2006).

Effects of soaking *Striga* seeds, pre-conditioned in water, in 2,4-D on attachment to sorghum roots

When *Striga* seeds pre-conditioned in water for 12 days and then soaked in water for 10-120 min., attachment of *Striga* germilings to sorghum seedling roots was very high (85-90%) (Table 2). In contrast, *Striga* seeds pre-conditioned in water and then soaked in 2,4-D at 20 or 80 □M for 10-120 min., attachment of *Striga* germilings progressively decreased with increased soaking period. Moreover, increasing the concentration of 2,4-D from 20 to 80 □M decreased attachment at all soaking periods. Maximum attachment (35%) was attained at soaking in 20 □M 2,4-D for 10 min, while the minimum (7.3%) was achieved at soaking in 80 □M 2,4-D for 120 min. Witchweed seeds were reported to become soft at the reticular end 3-6 h after stimulant treatment (Egley, 1972). Softening of the seed coat and consequently rapid penetration of the herbicide led to a toxic effect (Dafaallah, 2006).

It would appear that the influence of 2,4-D on *Striga* parasitism is complex. The herbicide could inhibit haustorium initiation and hence attachment to the host. Interference of 2,4-D with haustorium initiation is consistent with the observed reduced attachment of the parasite to sorghum.

Table 2. Effects of soaking *Striga* seeds pre-conditioned in water and 2,4-D concentration on attachment to sorghum roots

Soaking time (min.)	Haustorium attachment (%) 2,4-D concentration (□M)		
	0	20	80
10	90 a	35.0 a	18.0 a
	(72.4)	(36.5)	(25.2)
15	85 ab	30.7 b	16.0 ab
	(67.9)	(33.8)	(23.5)
30	84.5 b	23.7 c	11.0 bc
	(67.8)	(29.2)	(19.5)
60	89.5 a	19.3 d	8.7 c
	(72.0)	(26.2)	(16.8)
120	90 a	15.0 e	7.3 c
	(72.0)	(22.9)	(15.8)
S.E±	1.34	0.79	1.35
CV (%)	3.3	4.6	11.6

* Data between parentheses are arcsine transformed.

* Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

CONCLUSIONS

Haustorium induction of witchweed and hence its attachment to sorghum seedling roots was increased with concentration of DMBQ (2.5 and10.0 □M) and sorghum root extract (6.3 and 25 g/L). However, haustorium induction was progressively and significantly suppressed with increased 2,4-D concentration (2.5-100□M) and soaking period (10-120 min). Much remains to be learnt about the action of 2,4-D whitch appears to have a potential for witchweed management. However, further work is needed to clear the basic principles of 2,4-D actions on witchweed.

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تأثير مبيد **2,4-D** و **DMBQ** ومستخلص جذور الذرة على نشؤ المماص والتصاق طفيل البُودا **[*Striga hermonthica* (Del.) Benth.]** بجذور الذرة **[*Sorghum bicolor* (L.) Moench]**

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الخلاصة

أجرى نوعان من التجارب المعملية خلال أبريل 2006 في واد مدني، السودان. أجريت التجربة الأولى لدراسة تأثير مبيد 2,4-D بتركيز 2.5-100 مايكرومولر ومبدئ نشوء المماص (2,6-dimethoxy-ρ-benzoquinone) بتركيز 2.5 و10 ميكرومولر ومستخلص جذور الذرة بتركيز 6.3 و 25 جم/لتر على نشوء مماص (Haustorium) طفيل البودا [*S. hermonthica* (Del.) Benth.]. التجربة الثانية أجريت لدراسة تأثير مبيد 2,4-D بتركيز 20 أو 80 ميكرومولر لتهيئة بذور طفيل البودا ونقع البذور التي تمت تهيئتها في ذات التراكيز لمدة 10- 120 دقيقة على التصاق مماص الطفيل بجذور بادرة الذرة. وضعت المعاملات في تصميم عشوائي كامل بثلاث تكرارات. اختبرت بادرات البودا بغرض نشوء مماص والالتصاق بعد 4 و15 يوماً من المعاملة، على التوالي. جمعت البيانات حولت (arcsine) عند الحوجة، ثم أخضعت لتحليل التباين (ANOVA). تمت مقارنة المتوسطات بواسطة اختبار دنكن (DMRT)، عندما كان الاختبار معنوياً. أوضحت النتائج أنه انخفض تطور المماص والتصاقه بجذور بادرة الذرة بزيادة تركيز مبيد 2,4-D. إلاّ أن نشوء المماص ارتفع عند زيادة تركيز مبدئ نشوء المماص (2,6-dimethoxy-ρ-benzoquinone) ومستخلص جذور الذرة.