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The effect of nitrogen on the growth and development of giant witchweed, *Striga hermonthica* Benth.: effect on cultured germinated seedlings in host absence

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Abstract

For the first time in sterile nutrient media in the absence of the host plant, different forms and rates of nitrogen compounds were screened for their effect on *S. hermonthica* Benth. shoot development beyond seed germination. There was no shoot formation beyond the inoculation stages when *S. hermonthica* germlings were grown in media devoid of nitrogen source. In culture media containing some nitrogen sources, healthy shoots were formed. Increasing concentrations of KNO_3, $NaNO_3$, $Ca(NO_3)_2$, $Mg(NO_3)_2$ and asparagine resulted in a significant increase in *S. hermonthica* shoot development. Inversely, increasing concentrations of $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4Cl and urea led to increasing significant reduction of *S. hermonthica* shoot development. The amino acids, glycine and asparagine supported reduced shoot development of *S. hermonthica*, indicating that organic nitrogen cannot replace inorganic nitrogen for *Striga* growth. The ammonium nitrogen compounds, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$, suppressed further shoot elongation and total dry weight of 20 and 40 days old *Striga hermonthica* plants, in sterile culture media. The organic compounds urea, allylthiourea and thiourea had an effect similar to ammonium compounds. Arginine and glycine on the other hand did not suppress the further development of the parasite. The suppressive effect of nitrogen however, was greater when the parasite was 20 days old than when it was 40 days old. This work provides data to show that some nitrogen compounds reduce the severity of *S. hermonthica* attack by direct suppression of *Striga* growth and development at the post-germination stage and after shoots have been formed.

Introduction

Unemerged *Striga hermonthica* Benth. (Scrophulariaceae) seedlings, depend on the host plant for all nutritional requirements (holoparasites). On emergence, *S. hermonthica* becomes an obligate hemiparasite, with the ability to manufacture part of its nutritional requirements through photosynthesis. *Striga* spp. have been reported to cause more damage to the host plant when under the soil than when emerged [Sauerborn, 1991]. Due to the virulent effect of *Striga* spp. on the cultivated host plants, different control methods e.g. use of nitrogen fertilizers, have been identified and are gradually being improved upon.

It is already known that nitrogen reduces the severity of *Striga* attack while increasing the host yield simultaneously [Agabawi and Younis, 1965; Ogborn, 1984]. However, the mode of action of nitrogen on *Striga* has not been well understood [Ramaiah, 1984]. It is unclear whether the beneficial effect of nitrogen is due only to improved host vigour or also to suppression of parasite development. Does nitrogen inhibit the parasite plant development directly, or does it strengthen the maize host so that it can effectively withstand the parasite attack [Okonkwo, 1991]? One of the difficulties encountered in answering these questions has been to separate the response of the host from that of the parasite. Since the parasite and host grow together in the soil, it is not possible to isolate these factors

in field studies. Only during the initial germination stage of the parasite are the host and parasite separate for a short time, and it has been demonstrated that some nitrogen compounds inhibit germination and radicle extension growth of *Striga* seeds [Pesch and Pieterse, 1982; Okonkwo, 1991; Cechin and Press, 1993; Igbinnosa, 1993]. Little is know about the forms of nitrogen which are effective in suppressing *Striga* plant development. Besides no data currently exists on whether nitrogen compounds also suppress *S. hermonthica* seedlings after shoot formation, directly. The use of the aseptic culture technique as a tool for studying the mode of development, nutrition and reproduction of parasitic weeds for effective control, was first proposed by Cutter [1955]. Eventually, an aseptic culture technique for growing *Striga* plants was developed by Okonkwo [1966a, b]. This technique is ideal for assessing the effect of chemicals and herbicides on *Striga* spp. development, in the absence of the host plant. It is hoped that these findings would assist in the design of future experiments using nitrogen fertilizers and in the design of nitrogenous systems for use by farmers for the control of *Striga* in the field.

Materials and methods

Seeds of *S. hermonthica* were collected in 1976 from a sorghum farm in Samaru, Zaria, Kaduna State, Nigeria. These seeds were dried, put in small corked vials and kept in the dessicator at 25±2 °C over $CaCl_2$ until required.

Preconditioning and germination of S. hermonthica *seeds*

Method of culturing germinated embryos of *S. hermonthica* was adapted from Okonkwo [1964, 1966a, b; Igbinnosa, 1993]. Seeds of *S. hermonthica* were sieved to remove as much debris as possible. Then surface-sterilized by immersion in 1% sodium hypochlorite solution containing 2 to 3 drops of Tween-20, for 10 to 15 min in a sterile 50 ml beaker. Sterile *S. hermonthica* seeds were then rinsed 3 times with sterile distilled water to remove chlorine. The sterile *S. hermonthica* seeds were transferred into 6 cm diameter Petri dishes containing 10 to 15 ml of sterile distilled water, and transferred into the dark Percival (brand) incubator set at 25 °C. After 8 days preconditioning, the sterile distilled water was gently sucked out of the Petri dish using a sterile hypodermic syringe, with needle. The distilled water was replaced with 10 ml of 1 mg l^{-1} GR-24 [Johnson *et al.*, 1972], previously autoclaved at 121 °C, 105.46 kg/cm^2 pressure for 15 min, and cooled. The Petri dishes were transferred to the dark incubator at 25 °C for another 2 to 3 days, after which germination occurred.

Preparation of culture media

The culture media consisted of Murashige and Skoog [1962] mineral salts, 20 mg l^{-1} sucrose (source of carbon), and 9 gm l^{-1} of purified Difco Bacto agar. The nitrogen compounds contained in Murashige and Skoog (MS) mineral salts namely, NH_4NO_3 and KNO_3 (total nitrogen = 0.06 M), were replaced with the test nitrogen compounds. Culture media containing complete MS mineral salts served as reference point and control.

Nitrogen compounds screened at 0.0001 M, 0.0005 M, 0.001 M, 0.005 M, 0.01 M, 0.03 M and 0.06 M concentrations of compound include, NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, $NaNO_3$, KNO_3, $NaNO_2$, KNO_2, urea, allylthiourea, thiourea, glutamine, asparagine, glycine and arginine. The nitrogen compounds contained in MS mineral salts namely, NH_4NO_3 and KNO_3, were replaced with the test nitrogen compounds.

In a measuring cylinder, one litre of culture media was prepared and the pH adjusted to between 5.6 and 5.8. Into 200×25 mm culture tubes with air-tight screw-caps, were added 20 ml of the culture media, and the media were autoclaved as described above and slants were made on the laboratory bench.

Transfer of S. hermonthica *seedlings onto culture media*

Sterile geminated *S. hermonthica* seedlings and GR-24 contained in Petri dishes were poured into fresh sterile 9.5 cm diameter Petri dishes, each containing one layer of filter paper to facilitate removal of germination stimulant without losing some seeds, clearer view under the microscope and easy transfer of *Striga* germlings. The GR-24 contained in each Petri dish was removed using a sterile hypodermic syringe with needle. Under the microscope, and using sterile mounted needle, *S. hermonthica* germlings were individually transferred to the surface of the culture media contained in culture tubes. Each treatment had 12 replicate culture test tubes with each tube containing 2–3 *S. hermonthica*

Table 1. Effect of ammonium and nitrate nitrogen compounds on the development of *S. hermonthica* plant grown in culture media after 40 days in the dark, at 25 °C. Values are means of 22–24 plants each. MS = control media with Murashige and Skoogs' mineral salts containing 0.04 M NO_3^- and 0.02 M NH_4^+ ion

Treatment	Concentration (molarity)							
	MS	0	0.0005	0.001	0.005	0.01	0.03	0.06
	Shoot length (mm)							
$(NH_4)_2SO_4$		0.0	23.2	14.1	11.9	32.0	7.10	5.70
$NH_4H_2PO_4$		0.0	17.3	16.1	20.8	9.8	0.0	0.0
NH_4NO_3		0.0	26.9	23.7	27.1	21.4	35.5	25.9
NH_4Cl		0.0	19.3	21.4	28.2	11.7	6.1	3.9
KNO_3		0.0	0.0	0.0	3.5	4.4	8.4	8.4
$NaNO_3$		0.0	0.0	0.0	4.3	4.7	8.3	11.6
$Ca(NO_3)_2$		0.0	3.0	1.5	7.7	7.4	10.8	13.7
$Mg(NO_3)_2$		0.0	2.7	0.0	12.3	9.9	8.9	5.8
MS	27.7							

L.S.D. (p = 0.05) of Treatment × Concentration = 11.7

Table 2. Effect of ammonium and nitrate nitrogen compounds on the development of *S. hermonthica* plant grown in culture media after 40 days in the dark, at 25 °C. Values are means of 22–24 plants each. MS = control media with Murashige and Skoogs' mineral salts containing 0.04 M NO_3^- and 0.02 M NH_4^+ ion

Treatment	Concentration (molarity)							
	MS	0	0.0005	0.001	0.005	0.01	0.03	0.06
	Total dry weight (mg)							
$(NH_4)_2SO_4$		0.0	3.2	2.7	2.1	2.5	3.4	1.3
$NH_4H_2PO_4$		0.0	3.1	3.2	2.5	1.6	1.1	0.0
NH_4NO_3		0.0	5.2	4.8	5.7	4.1	4.7	6.1
NH_4Cl		0.0	2.3	3.7	2.5	3.5	2.2	1.7
KNO_3		0.0	0.0	0.0	0.6	0.9	1.2	2.2
$NaNO_3$		0.0	1.3	2.5	2.1	2.5	2.6	2.9
$Ca(NO_3)_2$		0.0	0.5	0.5	1.8	1.2	2.1	3.8
$Mg(NO_3)_2$		0.0	0.8	1.6	1.6	2.1	2.6	2.2
MS	4.6							

L.S.D. (p = 0.05) of Treatment × Concentration = 1.2

seedlings. All operations were conducted under aseptic conditions in Laminar-Flow-Hood.

The culture tubes were transferred to a dark Conviron (brand) growth chamber set at 25 °C. After 40 days, the *S. hermonthica* seedlings were removed from the culture tubes using forceps and the shoot lengths were measured using a mm ruler. The seedlings were dried in an oven, for 48 h at 80–85 °C, cooled and total dry weights were measured.

Effect of nitrogen compounds on 20 and 40 days old S. hermonthica *plants*

In another set of experiments, *S. hermonthica* seedlings were grown in sterile media containing complete MS mineral salts. After 20 and 40 days growth respectively, *S. hermonthica* seedlings were removed from the culture tubes using sterile forceps and transferred into fresh aseptic media containing 0.03 M and 0.06 M $(NH_4)_2SO_4$, $NH_4H_2PO_4$ or 0.01 M and 0.03 M urea, thiourea, allylthiourea, glycine and arginine, respectively. After a further 14 days, making a total of 34 and 54 days respectively, the shoot lengths and total dry weights were measured as described above. Plantlets grown for 20 and 40 days respectively in MS media and transferred into fresh MS media for a further 14 days, served as controls.

Comparison of some ammonia compounds with non-ammonia sodium compounds

In a separate experiment, the ammoniated compounds, NH_4Cl and $(NH_4)_2SO_4$ were compared with the non-ammoniated sodium compounds NaCl and Na_2SO_4 with the same anions, to find out whether the suppression of *S. hermonthica* shoot development was solely by NH_4^+. The compounds were screened at 0.005 M in modified media already containing 0.03 M NH_4NO_3. The conditions of growth are similar to that described above. All experiments were repeated at least once. Values presented are means of 22 to 24 plants each, and to check for significant differences between means, the Least Significant Difference (L.S.D.) test was carried out.

Table 3. A comparison of some ammonium compounds with non-ammoniated sodium compounds on the shoot development of *S. hermonthica* plant grown in culture media after 40 days in the dark, at 25 °C. Compounds were screened at 0.005 M in media already containing 0.03 M NH_4NO_3. Control is media containing 0.03 M NH_4NO_3 only. Values are means of 22–24 plants each

Treatment	Shoot length (mm)	Total dry weight (mg)
Control	30.5	4.1
NH_4Cl	19.2	2.5
NaCl	35.1	4.4
$(NH_4)_2SO_4$	8.9	2.3
Na_2SO_4	34.2	6.8
L.S.D. ($p = 0.05$) =	4.1	0.8

Results

In culture media devoid of nitrogen source, *S. hermonthica* seedlings hardly developed beyond the inoculation stage (data not shown). In control MS media, healthy *S. hermonthica* plants developed, with 27.7 mm average shoot length and 4.6 mg average total dry weight, after 40 days.

With increasing concentration of $NH_4H_2PO_4$ from 0.0005 M, the shoot length and total dry weight of *S. hermonthica* plants decreased significantly until 0.06 M when zero plant growth was recorded (Tables 1 and 2). The nitrogen compounds, $(NH_4)_2SO_4$ and NH_4Cl significantly suppressed *S. hermonthica* shoot length and total dry weight, with increasing concentration from 0.0005 M to 0.06 M, and compared with the control. NH_4NO_3 did not suppress the shoot length and total dry weight of *S. hermonthica* plants at any concentration, compared with the control (Tables 1 and 2). To check for anion effect, some ammonium compounds were screened along with non-ammoniated sodium compounds with the same anions. Results clearly show high significant suppression of *S. hermonthica* plant development by ammoniated compounds, but not non-ammoniated sodium compounds, compared with control. For example, average shoot length of *S. hermonthica* seedlings was 19.2 mm and 35.1 mm in NH_4Cl and NaCl respectively (Table 3).

As nitrate compounds concentration namely, KNO_3, $NaNO_3$, $Ca(NO_3)_2$ and $Mg(NO_3)_2$ increased, the shoot length and total dry weight of *S. hermonthica* plant increased. This increase was not significant at the 5% level of probability (Tables 1 and 2). It is noted that highest *S. hermonthica* plant growth in nitrate source, 0.06 M $Ca(NO_3)_2$, was significantly lower than MS control containing both ammonium and nitrate ions. No further *S. hermonthica* development beyond radicle emergence occurred when nitrite compounds, KNO_2 and $NaNO_2$ were applied (data not presented).

With increase in urea concentration from 0.0001 M, *S. hermonthica* shoot length increased until 0.0005 M concentration. After 0.0005 M urea concentration, *S. hermonthica* shoot length declined significantly until 0.03 M concentration when 0 mm was recorded. It is noted that 0.0005 M urea significantly suppressed *S. hermonthica* shoot length, compared with the control (Table 4). At lower concentrations of 0.0005 to 0.005 M urea, the total dry weight of *S. hermonthica* seedling was not significantly different from inorganic control. However, at higher concentrations of 0.01 M to 0.06 M urea, *S. hermonthica* total dry weight was significantly lower than the control. There were little or no *S. hermonthica* shoot development beyond radicle emergence when thiourea and allylthiourea were applied (Table 5).

Glutamine, glycine and arginine, shoot length of *S. hermonthica* plants decreased as their concentrations increased. In general, *S. hermonthica* plants performed poorly in these organic compounds compared with inorganic MS control. However, at 0.0005 M glutamine concentration, shoot length was not significantly different from inorganic control. *S. hermonthica* shoot length increased with increase in asparagine concentration (Table 4). The same trend described above is seen for *S. hermonthica* total dry weight (Table 5).

Table 4. Effect of organic nitrogen compounds on the development of *S. hermonthica* plant grown in culture media after 40 days in the dark, at 25 °C. Values are means of 22–24 plants each. MS = control media with Murashige and Skoogs' mineral salts containing 0.04 M NO_3^- and 0.02 M NH_4^+ ion

Treatment	Concentration (molarity)								
	MS	0	0.0001	0.0005	0.001	0.005	0.01	0.03	0.06
	Shoot length (mm)								
Urea		0.0	7.5	15.5	11.3	11.8	3.5	0.0	0.0
Thiourea		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Allylthiourea		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glutamine		0.0	0.0	26.3	14.1	10.1	5.8	4.0	3.4
Glycine		0.0	0.0	0.0	0.0	4.5	0.0	0.0	0.0
Asparagine		0.0	0.0	5.0	6.6	10.6	6.6	26.8	10.0
Arginine		0.0	0.0	3.6	4.9	3.0	0.0	0.0	0.0
MS	27.7								

L.S.D. ($p = 0.05$) of Treatment × Concentration = 6.2

Table 5. Effect of organic nitrogen compounds on the development of *S. hermonthica* plant grown in culture media after 40 days in the dark, at 25°C. Values are means of 22–24 plants each. MS = control media with Murashige and Skoogs' mineral salts containing 0.04 M NO_3^- and 0.02 M NH_4^+ ion

Treatment	Concentration (molarity)								
	MS	0	0.0001	0.0005	0.001	0.005	0.01	0.03	0.06
	Total dry weight (mg)								
Urea		0.0	2.3	4.1	4.9	7.6	0.5	0.0	0.0
Thiourea		0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0
Allylthiourea		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glutamine		0.0	1.2	4.9	1.8	2.5	1.6	2.4	1.3
Glycine		0.0	0.3	0.6	0.4	0.7	1.0	0.8	0.0
Asparagine		0.0	2.1	1.3	1.9	2.4	2.7	4.5	2.9
Arginine		0.0	0.4	0.7	0.8	0.6	0.5	0.0	0.0
MS	4.6								

L.S.D. ($p = 0.05$) of Treatment × Concentration = 1.5

Effect of nitrogen compounds on 20 days old S. hermonthica *seedlings*

The average shoot length and total dry weight of the control *S. hermonthica* plantlets, after 20 days in MS media were 5.9 mm and 1.1 mg respectively. On transfer onto fresh control MS media for further 14 days, the average shoot length and total dry weights significantly increased to 13.1 mm and 4.2 mg respectively (Tables 6 and 7). After 14 days in media containing 0.03 M $(NH_4)_2SO_4$, shoot length of *S. hermonthica* seedlings was significantly higher than 20 days old control, but significantly lower than 34 days old control of the same age. At 0.06 M $(NH_4)_2SO_4$, *S. hermonthica* plants average shoot length was not significantly different from 20 days old control (Table 6). *S. hermonthica* shoot length in both 0.03 M and 0.06 M $NH_4H_2PO_4$ concentrations was not significantly different from 20 days old control, but significantly different from 34 days old control (Table 6). At 0.03 M concentrations each, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ did not suppress the total dry weight of *S. hermonthica* plants compared with controls. However, a higher dosage of 0.06 M $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ concentrations significantly suppressed *S. hermonthica* plant dry weights compared with the 34-day old control (Table 6).

At 0.01 M and 0.03 M urea, thiourea and allylthiourea, the further growth of 20 days old *S. hermonthica* plants were significantly suppressed compared with 34 days old control (Table 7). Glycine appears to have supported further *S. hermonthica* shoot growth

Table 6. Effect of two weeks exposure to ammonium compounds on the development of 20 days *Striga hermonthica* plants grown *in vitro*. Values are means of 22–24 plants each. **20d (MS)** = Seedlings grown for 20 days in full MS medium. **34d (MS)** = Seedlings grown for 20 days in full MS medium and transferred to fresh MS medium for further 14 days

Treatments	Shoot length (mm)			Total dry weight (mg)		
		Concentration (M)			Concentration (M)	
		0.03	0.06		0.03	0.06
20d (MS)	5.9	–	–	1.1	–	–
34d (MS)	13.1	–	–	4.2	–	–
$(NH_4)_2SO_4$	–	10.3	4.9	–	4.1	2.7
$NH_4H_2PO_4$	–	6.4	5.3	–	5.4	1.5

L.S.D. (p = 0.05)
Treatment × Shoot length = 1.4
Treatment × Total dry weight = 0.7

Table 7. Effect of two weeks exposure to organic compounds on the shoot development of 20 days *Striga hermonthica* plants grown *in vitro*. Values are means of 22–24 plants each. **20d (MS)** = Seedlings grown for 20 days in full MS medium. **34d (MS)** = Seedlings grown for 20 days in full Ms medium and transferred to fresh MS medium for further 14 days

Treatments	Shoot length (mm)			Total dry weight (mg)		
		Concentration (M)			Concentration (M)	
		0.01	0.03		0.01	0.03
20d (MS)	5.9	–	–	1.1	–	–
34d (MS)	13.1	–	–	4.2	–	–
Urea	–	10.4	7.6	–	3.3	1.3
Thiourea	–	10.5	9.4	–	3.9	3.0
Allylthiourea	–	9.0	9.1	–	2.0	1.9
Glycine	–	19.8	16.5	–	5.5	4.4
Arginine	–	17.7	8.7	–	4.3	3.3

L.S.D. (p = 0.05)
Treatment × Shoot length = 1.3
Treatment × Total dry weight = 0.4

Table 8. Effect of two weeks exposure to ammonium compounds on the development of 40 days *Striga hermonthica* plants grown *in vitro*. Values are means of 22–24 plants each. **40d (MS)** = Seedlings grown for 20 days in full MS medium. **54d (MS)** = Seedlings grown for 20 days in full MS medium and transferred to fresh MS medium for further 14 days

Treatments	Shoot length (mm)			Total dry weight (mg)		
		Concentration (M)			Concentration (M)	
		0.03	0.06		0.03	0.06
40d (MS)	13.1	–	–	4.2	–	–
54d (MS)	27.6	–	–	9.8	–	–
$(NH_4)_2SO_4$	–	31.3	27.3	–	7.9	5.8
$NH_4H_2PO_4$	–	20.1	17.8	–	5.8	3.0

L.S.D. (p = 0.05)
Treatment × Shoot length = 2.8
Treatment × Total dry weight = 1.2

Table 9. Effect of two weeks exposure to organic compounds on the development of 40 days *Striga hermonthica* plants grown *in vitro*. Values are means of 22–24 plants each. **40d (MS)** = seedlings grown for 20 days in full MS medium. **54d (MS)** = Seedlings grown for 20 days in full MS medium and transferred to fresh MS medium for further 14 days

Treatments	Shoot length (mm)			Total dry weight (mg)		
		Concentration (M)			Concentration (M)	
		0.01	0.03		0.01	0.03
20d (MS)	13.1	–	–	4.2	–	–
34d (MS)	27.6	–	–	9.8	–	–
Urea	–	21.7	13.4	–	7.9	6.5
Thiourea	–	29.9	26.9	–	9.4	6.7
Allylthiourea	–	34.4	29.1	–	3.1	5.3
Glycine	–	38.1	34.5	–	11.2	7.9
Arginine	–	21.6	17.2	–	9.2	6.7

L.S.D. (p = 0.05)
Treatment × Shoot length = 2.4
Treatment × Total dry weight = 0.7

compared to control, at both 0.01 M and 0.03 M concentrations, while arginine only suppressed *S. hermonthica* development at a higher dosage of 0.03 M concentration (Table 7).

Effect of nitrogen on 40 days old S. hermonthica *seedlings*

Average shoot length and total dry weight of *S. hermonthica* plantlets after 40 days in control MS media, were 13.1 mm and 4.2 mg respectively. On transfer into new control MS media for further 14 days, the average shoot length and total dry weight significantly increased to 27.6 mm and 9.8 mg respectively (Table 8). After 40 days in MS and then transfer into media containing 0.03 M and 0.06 M $(NH_4)_2SO_4$ for 14 days, the shoot length was not suppressed compared with 54 days old control. However, there was significant reduction in the total dry weight at both 0.03 M and 0.06 M $(NH_4)_2SO_4$ concentrations compared with 54 days old control. At 0.03 M and 0.06 M $NH_4H_2PO_4$, *S. hermonthica* shoot length and total dry weight were not suppressed compared to 40 days old control, however it was significantly suppressed compared with 54 days old control (Table 8).

Table 10. Conversion of Molar concentrations of $(NH_4)_2SO_4$ and urea into equivalents in KgN ha^{-1}

Nitrogen compounds	Concentration (molarity)	% soil-water content			
		10	20	40	80
		Equivalent in KgN ha^{-1}			
$(NH_4)_2SO_4$	0.06	63.0	126.0	252.1	345.7
	0.03	31.5	63.0	126.0	252.0
	0.01	10.5	21.0	42.0	84.0
	0.005	5.3	10.6	21.2	42.4
	0.001	1.1	2.2	4.4	8.8
	0.0005	0.5	1.0	2.0	4.0
Urea	0.03	126.0	252.0	504.0	1008.1
	0.01	42.0	84.0	168.0	336.0
	0.005	21.0	42.0	84.0	168.0
	0.001	4.2	8.4	16.8	33.6
	0.0005	2.1	4.2	8.4	16.8
	0.0001	0.4	0.8	1.6	3.2

Urea significantly suppressed the growth of 40 days old *S. hermonthica* seedlings at both 0.01 M and 0.03 M concentrations. Arginine had a similar effect, except at 0.01 M concentration where the total dry weight of *S. hermonthica* was not suppressed (Table 9). *S. hermonthica* plants grown in culture media containing 0.01 M and 0.03 M thiourea, allylthiourea and glycine did not reduce the parasite shoot length compared to the controls. Allylthiourea reduced the total dry weight of *S. hermonthica* compared with control, at both concentrations tried, while total dry weight loss was recorded only at a higher dosage of 0.03 M thiourea and glycine (Table 9).

Discussion and conclusion

That *S. hermonthica* seedlings cannot develop shoot in media devoid of nitrogen has been confirmed by this study [Okonkwo, 1966a, b]. It was also shown that as the concentration of some ammonium compounds increased, the shoot development of *S. hermonthica* decreased. It had been reported that ammonia does not accumulate in plant cells, with glutamine synthetase ensuring its elimination as an ionic species [Miflin and Lea, 1976]. An accumulation of ammonium ion in cells is toxic for plant growth [Givan, 1979]. The proton carried by the NH_4^+ ion must be eliminated from the cell cytoplasm [Raven, 1985]. Based on the above findings, it is possible that ammonium ion accumulated in *S. hermonthica* plants thus interfering with certain growth factors/substances contained in *S. hermonthica* required for continuous good growth. These growth factors may include proper and adequate moisture and nutrient uptake, efficient photosynthetic apparatus and right amount of growth hormones.

Thalouarn *et al.* [1987, 1988, 1990] had shown that ammonium assimilation in related *Lathraea clandestina* L. (Scrophulariaceae), takes place mainly through the glutamine synthetase/glutamate synthetase pathways, with glutamine synthetase activity in different organs of *L. clandestina* lower, compared with leaves of non-parasitic tobacco (*Nicotiana tabacum* L.) and maize (*Sea mays* L.) plants. Similarly, McNally and Stewart [1987] reported low glutamine synthetase activities in *Striga* spp. and other related parasitic plant species. It is possible that at higher concentrations, the accumulation of ammonia in cells of *S. hermonthica* plants is enhanced by the inability of glutamine synthetase to cope with its elimination leading to an accumulation of protons carried by NH_4^+ ion resulting in reduced growth and development in culture. It may be interesting to check for glutamine synthetase activity as well as NH_4^+ accumulation at various levels of ammonium compound application, in *Striga*. Also, it would be informative to check for the effect of NH_4^+ accumulation on cell pH level and on accumulation of salts in *Striga* spp.

Unlike nitrates, ammonia absorption and transportation through the plant cell plasmalemma is a passive process [Salsac *et al.*, 1987]. Besides, the supply of reduced nitrogen in form of NH_4^+ should make the biosynthesis of macro-molecules more economical, thereby leading to increased growth of the plant. The opposite however, has occurred with *S. hermonthica* since, ammonium supply inhibited the further development of *S. hermonthica* plants by between 40 to 70%.

The reason for increased shoot development observed when *S. hermonthica* was grown in NaCl and Na_2SO_4 but not NH_4Cl and $(NH_4)_2SO_4$, is not clear. It is possible that the Na^+ cation, but not NH_4^+ ion, may have increased the solute level in *S. hermonthica* cells resulting in increased water uptake, suppression of growth inhibitors and consequently better shoot development. Choic *et al.* [1961] observed a decrease in solute concentrations in the cell vacuoles of tomato plants (*Lycopersicum esculentum* L.) by ammonium nutrition, which led to insufficient water nutrition and invariably poor growth. This decrease in solute concentration was not compensated for by

increase in phosphate, sulphate and chloride levels always observed in ammonium nutrition. The presence or increase in the concentration of the Cl^-, SO_4^{2-} or $H_2PO_4^-$ anions did not compensate for solute deficiency in *S. hermonthica*. Whatever the case, it is clear from these findings that the toxic effect of ammoniated compounds on *S. hermonthica* growth is due to the ammonium ion and not the anion.

As the concentration of nitrates increased up to 0.06 M, *S. hermonthica* shoot development increased as well, suggesting that *S. hermonthica* may be able to utilize nitrate compounds at concentrations higher than 0.06 M, in culture. Unlike ammonia, the absorption of nitrate is an active process and it has been shown that nitrates accumulate rapidly and in large quantities in the vacuole of plant cells without being toxic to the plant [Martinoia *et al.*, 1981]. Nitrate nutrition also leads to the accumulation of solutes in plant cells resulting in increased water uptake and transportation in plant tissue [Salsac *et al.*, 1987], and thus increased growth. Simier *et al.* [1993] reported that inorganic ions such as K^+, Na^+ and Cl^- account for the greatest part of the osmolarity in unstressed *Thesium humile* Vahl. It is clear therefore that *S. hermonthica* plants can tolerate and better utilize nitrate sources than ammonium sources for their growth and development in culture media.

Hunter and Visser [1986] reported significant Nitrate Reductase Activity (NRA) increase in both *Alectra vogelii* Benth. and *S. asiatica* (L.) Kuntze. upon nitrate application. Steward *et al.* [1984] however failed to find any NRA increase following nitrate application to excised shoots of *S. asiatica* and *S. hermonthica*. It is possible that increasing concentrations of nitrate compounds induced increased NRA, enabling better utilization of nitrogen for *S. hermonthica* growth.

It is however noted that at higher concentrations of nitrate compounds, the shoot length of *S. hermonthica* was significantly less than the control containing both NNH_4NO_3 and KNO_3. It is possible that NRA was not induced at a sufficient quantity to ensure adequate utilisation of nitrates for better growth of *S. hermonthica*, suggesting that the mechanism for induction of nitrate reductase (NR) is very poorly developed in *S. hermonthica* plants. Or that the presence of tolerable ammonium ion combined with nitrate, enhances NR production in *S. hermonthica* in an unknown way, ensuring better growth. It was not surprising therefore that the compound that least suppressed the shoot development of *S. hermonthica* was NNH_4NO_3. Interestingly, a ratio 1:1 NH_4^+/NO_3^- ions is required to overcome suppression by the NH_4^+ ion and achieve greater growth of plants. It would be worthwhile to investigate the effect of NH_4^+/NO_3^- ratios on the growth, and nitrogen assimilation enzyme regulation in *Striga* spp. Based on aseptic media studies, nitrate nitrogen and/or ammonium/nitrate fertilizers may be useless in suppressing the virulent effects of *S. hermonthica* on cultivated crops. However, Cechin and Press [1993] reported the suppression of the early growth of *S. hermonthica* seedlings attacking sorghum by higher NH_4NO_3 concentration.

It has been shown that NR is encoded for by two important genes, *nia*-1 and *nia*-2, which are responsible for the production of NR in higher plants [Crawford and Campbell, 1990]. It would be interesting to find out if these two genes are present in *S. hermonthica*, and then determine the efficiency of NR apparatus in assimilating nitrogen. This would help to explain why nitrate assimilation and consequently shoot growth of *S. hermonthica* is relatively poor in culture especially at lower concentrations.

In this study, $(NH_4)_2SO_4$, $NH_4H_2PO_4$ and urea suppressed *S. hermonthica* shoot development at the post-germination stages and after 20 and 40 days growth in aseptic media, and at concentrations that can be applied in the field (Table 10). Nitrogen compounds suppressed further plant development more at the younger stage of *S. hermonthica* growth than at older stage, suggesting that the influence of nitrogen on the physiological growth processes within the parasite declines as it gets older. The reason for this is unknown. Therefore the application of nitrogen fertilizers such as $(NH_4)_2SO_4$, $NH_4H_2PO_4$ and urea, before, and immediately after shoot formation, that is the younger stages of the parasite development, would ensure better control of the parasite. Okonkwo [1991] reported the reduction of *S. asiatica* seedlings at the post-germination stage in sterile media, by urea. Kroschel [1989] also reported the suppression of *S. hermonthica* seedlings growth and development by $(NH_4)_2SO_4$ and urea in the presence of host sorghum.

The inhibition of *S. hermonthica* shoot in culture by urea was not surprising as it is known that urea is converted to ammonium ion in most higher plant cells, before assimilation [Beevers, 1976]. This also probably explains the effects of thiourea and allylthiourea as well. Glutamine and asparagine, on the other hand, can replace inorganic nitrogen at lower and higher concentrations, respectively. The amino amides glycine and arginine, cannot replace inorganic nitrogen as sources

of nitrogen for *S. hermonthica* growth. Simier *et al.* [1993] had previously shown that amino acids account for a small part of the osmolarity in unstressed *Thesium humile*. The reason why some organic compounds e.g. arginine and glycine, inhibit *S. hermonthica* shoot formation beyond radical emergence, but not suppress the further shoot development of *S. hermonthica* plant is not known.

In conclusion, it has been observed in the field that nitrogen fertilizers suppress the severity of *Striga* spp. attack while improving crop yield. How this happens was not known. Some researchers have provided data to show that, among other possibilities, nitrogen acts by reducing the percentage germination of *Striga* seeds in the presence of germination stimulants [Pesch and Pieterse, 1982; Okonkwo, 1991; Igbinnosa, 1993]. From this study, another mode of fertilizer action is that, some nitrogen compounds transferred from host xylem to parasite via the haustorium, directly suppress *S. hermonthica* growth and development by one or all of the reasons given above. The application of some nitrogen compounds e.g. $(NH_4)_2SO_4$, $NH_4H_2PO_4$ and urea to *Striga* infested fields, would reduce the severity of the parasite attack, even after emergence. Thus resulting in improved crop yield.

This is the first time that nitrogen nutrition on any *Striga* spp. has been studied under sterile conditions on a nutrient medium in the absence of a host. It is hoped that these contributions would improve confidence in nitrogen fertilizer use, and also assist in designing control packages for *Striga* in the field especially using the nitrogenous fertilizer system.

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