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# Impact of nitrate supply in C and N assimilation in the parasitic plant *Striga hermonthica* (Del.) Benth (Scrophulariaceae) and its host *Sorghum bicolor* L.

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## ABSTRACT

**The threshold of tolerance for nitrate of the parasitic weed *Striga hermonthica* (Del.) Benth and the host plant *Sorghum bicolor* L. was determined by estimating the impact of increasing nitrate loads on plant growth and various parameters of C and N assimilation. Nitrate supply improved chlorophyll (Chl) content and photosystem II (PSII) photochemistry of infected *S. bicolor* that, in comparison to *S. hermonthica*, displayed a low imbalance between C and N assimilation when nitrate was supplied up to 1500 mg N per plant. Indeed, nitrate supplies increased strongly the leaf N:C ratio of the parasite. The higher nitrate load induced strong accumulation of nitrate, nitrite and ammonium, and consequently the death of *S. hermonthica*. Nevertheless, lower nitrate loads (up to 500 mg N per *S. bicolor* in this study) promoted leaf expansion, PSII photochemistry and N metabolism of *S. hermonthica* mature (M) plants, as attested by the significant rise in soluble protein and free amino-acid contents. Following these N supplies, the nitrate tolerance of *S. hermonthica* was correlated with an increase in PSII activity and a high incorporation of N excess into asparagine. This confirmed the central role of asparagine in the N metabolism of *S. hermonthica*, although this detoxification pathway was insufficient to limit ammonium accumulation under higher nitrate loads.**

*Key-words*: ammonium; asparagine; chlorophyll *a* fluorescence; fertilization; nitrite; photochemistry.

*Abbreviations*: AS, asparagine synthetase [enzyme class (EC) 6.3.5.4]; Chl, chlorophyll; DM, dry matter; FAA, total free amino acid; $F_v/F_m$, maximal quantum yield of photosystem II photochemistry, calculated in the dark-adapted state; $F_0$, minimal fluorescence yield following dark adaptation (with all photosystem II centres fully open); $F_m$, maximal fluorescence yield in the dark-adapted state (with all photosystem II centres closed); $F'_0$, minimal fluorescence yield of a pre-illuminated sample (with all photosystem II centres fully open); $F'_m$, maximal fluorescence yield in the light-adapted state; $F_s$, steady-state fluorescence yield at a given measuring light intensity; FW, fresh weight; NR, nitrate reductase; PSII, photosystem II; PFD, photon flux density (400–700 nm); *qP*, photochemical quenching of fluorescence; wae, weeks after emergence above the ground; $\Phi_{PSII}$, efficiency of photosystem II photochemistry in the light-adapted state.

## INTRODUCTION

The parasitic weed *Striga hermonthica* (Del.) Benth (Scropulariaceae) causes a serious problem on maize and sorghum in Eastern, Central and Western Africa (Riches & Parker 1995). The parasite grows underground for about 4–6 weeks from host-derived resources, causing considerable damage to the infected plant, which displays dehydration, reduced photosynthetic performance and loss in both vigour and grain yield (Graves, Press & Stewart 1989; Graves 1995). After emergence, a dramatic transpiration rate maintains an intensive diversion of resources from the host xylem sap (Ackroyd & Graves 1997).

To attempt to reverse the spread of *S. hermonthica*, while an investigation of varietal resistance in sorghum is in progress (Haussmann *et al*. 2004), several integrated pest-management programmes have been proposed that combine agronomic practices (Hess *et al*. 2001; Marley *et al*. 2004), with chemical and biological control approaches (Babiker *et al*. 1996; Gworgwor & Weber 2003). The low N availability in the soil promotes *S. hermonthica* infection (Farina, Thomas & Channon 1985), thus N fertilization is recommended seeing that it improves crop yield and reduces *S. hermonthica* infestation (Osman, Raju & Peacock 1991; Singh, Ndikawa & Rao 1991). More precisely, a toxic effect of nitrate on subterranean *S. hermonthica* seedlings was considered by some authors (Cechin & Press 1993a; Eplee, Norris & Merritt 1994; Press & Cechin 1994;

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Pieterse 1996), but the mechanisms involved in N-related toxicity need clarification.

Regarding the benefit of N fertilization as a means of crop protection against *S. hermonthica*, some essential traits of N relationships between host and parasite and inorganic N assimilation in *S. hermonthica* have been investigated. Pageau *et al*. (2003) have shown that, under a nitrate-fertilization regime, the nitrate was absorbed by the host sorghum roots and transferred un-assimilated to *S. hermonthica* as the major host-derived N compound. Thus, *S. hermonthica* shoots displayed high N:C ratio and N sequestration into asparagine. This is compatible with the previous findings that NR is more active in *S. hermonthica* after emergence, that it is mainly in the leaves and is nitrate induced (Igbinnosa & Thalouarn 1996), and with recent evidence that the expression of the *ShAS* gene (encoding for AS, the primary enzyme involved in asparagine production) is strongly induced by light in the leaves of the parasite (Simier *et al*. 2005). Consequently, N toxicity is suspected to be lower once *S. hermonthica* has emerged and developed photosynthetic leaves. However, the extent to which the leaves are able to manage N excess following N fertilization needs clarification.

The positive effect of nitrate fertilization on development, photosynthesis and N assimilation is well known in plants (Stitt 1999). Here, the purposes were rather to estimate the respective impact of N fertilization on the infected *Sorghum bicolor* L. and its root-connected parasite *S. hermonthica*. Two questions require investigation: up to what load will nitrate fertilization favour the vigour of infected *S. bicolour*?; up to what nitrate load can the xylem-branched parasite *S. hermonthica* manage efficiently the nitrate influx by diverting nitrate–N into asparagine? These questions are addressed and investigated in the present study following C and N assimilation both in *S. bicolor* and *S. hermonthica*. Simultaneously, most of the experiments were performed on un-infected *S. bicolor* plants. While these results are not presented here in order to improve the visibility of the results concerning nitrate impact on host–parasite association, they are discussed because the threshold of tolerance of single *S. bicolor* to nitrate is essential in the agronomical field when nitrate supply is proposed to control *S. hermonthica*.

## MATERIALS AND METHODS

### Plant material and growth conditions

Plants were grown in a glasshouse under a 14 h photoperiod with a PFD of 300 $\mu$mol m$^{-2}$ s$^{-1}$ at 30/35 °C night/day. Seeds of *S. hermonthica* collected from Wad Madani (Sudan) were preconditioned for 7 d at a density of 10 mg dm$^{-3}$ in pots (Pageau *et al*. 2003). One *S. bicolor* var. SH4 Arval seed was placed in each pot containing preconditioned *S. hermonthica* seeds. The cultures were watered three times per week with distilled water. The age of the parasites was expressed in weeks from the date of emergence.

The development of *S. hermonthica* plants is known not to be synchronized. When the first parasites emerged in the fourth to sixth week after host sowing, the pots were distributed in six plastic trays containing 7 dm$^3$ of a sand-bed washed previously with abundant water. The number of *S. hermonthica* that emerged was relatively close in the selected pots (8–12 per pot). Four pots were placed in each tray. The *S. bicolor* roots that were free of *S. hermonthica* grew in the sand-bed, which was first supplied with nitrate when the first-emerged parasites were 3 wae. Each culture tray was supplied weekly for 2 weeks with 1.5 L of $KNO_3$ solution at various concentrations, resulting in nitrate loads of 25, 100, 200, 500 and 1500 mg N per *S. bicolor*.

### Leaf sampling

Two weeks after the last nitrate supply, the well-expanded leaves of *S. bicolor* and *S. hermonthica* were analysed at the fourth hour of photoperiod for Chl *a* fluorescence, $O_2$ exchanges and Chl content. Once these analyses were finished, all the other well-expanded leaves of both plants were dried at 80 °C for 24 h, then ground to a fine powder using a Retch ball mill (Brinkmann Instruments Inc., NY, USA). For *S. hermonthica*, the analyses were performed only on mature (M) 7-wae-old plants.

### Chl *a* fluorescence analysis

The Chl *a* fluorescence was analysed using a pulse-modulated fluorescence monitoring system (FMS 2, Hansatech Instruments Ltd, King's Lynn, UK). The plants were dark adapted for 2 h prior to $F_0$ measurements by applying far-red illumination (735 nm). $F_m$ was determined following a saturating light flash (18 000 $\mu$mol m$^{-2}$ s$^{-1}$, 0.4 s duration). $F_v$ was calculated as the difference between $F_m$ and $F_0$. $\Phi_{PSII}$ was calculated at a PFD of 293 $\mu$mol m$^{-2}$ s$^{-1}$ from the measurements of $F'_m$ and $F_s$ (steady state) in the light-adapted state, according to Genty, Briantais & Baker (1989). $F'_0$ was determined by applying far-red illumination after $F_s$ determination, and served to calculate $qP$.

### Total Chl content

Chl was extracted from 100 mg FW of leaves in 10 mL of 80% (v/v) acetone. After centrifugation (12 000 *g*, 10 min), the Chl content was determined spectrophotometrically using the extinction coefficients of Arnon (1949).

### $O_2$ exchange analysis

The $O_2$ evolution rates were measured polarographically in 10 mM 3-morpholinopropanesulfonic acid (MOPS)–KOH buffer (pH 7.2), under saturating $CO_2$ concentration (15 mM $NaHCO_3$) using an $O_2$ electrode (Hansatech Instruments Ltd). Net photosynthesis and respiration rates were measured at saturating PFD (1000 $\mu$mol m$^{-2}$ s$^{-1}$) and in darkness, respectively. PSII activity in $O_2$ production, assessed as the gross photosynthesis, was calculated as the sum of the net photosynthesis and the respiration rates.

 

### Determination of C isotope composition, total N and C contents and N:C ratio

Isotopic ($\delta^{13}C$) and elemental analyses were determined by isotopic ratio mass spectrometry using a FinniganMAT (Bremen, Germany) Delta E isotope ratio mass spectrometer coupled to a micromass elemental analyser (NA2100, Fisons Instruments Inc.), as described previously by Pageau *et al*. (2003). Samples of *c*. 5 mg, containing *c.* 2 mg C were analysed in triplicate. The relative contribution of the autotrophic C to the growth of the parasites was calculated as described previously (Press *et al*. 1987a; Pageau *et al*. 1998).

### Determination of soluble protein, total FAA and asparagine contents

Soluble proteins and FAA were extracted from 50 mg DM in 5 mL of 0.2 M NaOH. After homogenization (Ultra-Turrax (Jake & Kundel, IKA Labortechnik, Staufen, Germany), 24 000 tr $min^{-1}$ for $3 \times 15$ s) and centrifugation (12 000 *g*, 10 min), the soluble proteins and FAA were quantified in the supernatant as described by Bradford (1976) and Yemm & Cocking (1955), respectively.

Asparagine was extracted from 50 mg DM in 1 mL 10 mM phosphate buffer (pH 7.5) following incubation at 30 °C for 5 min. After centrifugation (12 000 *g*, 10 min), asparagine was quantified spectrophotometrically ($A_{340}$) in the supernatant as described by Vadez, Sinclair & Sarraj (2000). The reaction mixture (total volume: 2.9 mL, including extract) contained 2 mL of 14.5 mM phosphate buffer (pH 7.5), 100 $\mu$L of 600 mM $\alpha$-ketoglutarate, 100 $\mu$L of 10 mM NADH, 2.5 U asparaginase [enzyme class (EC) 3.5.1.1, Roche, Indianapolis, IN, USA], 5 U aspartate aminotransferase (EC 2.6.1.1, Sigma, St Louis, MO, USA) and 5 U malate dehydrogenase (EC 1.1.1.37, Sigma).

### Determination of nitrate, nitrite and ammonium contents

Inorganic N compounds were extracted from 50 mg DM in 5 mL of ultra-pure water following incubation at 100 °C for 20 min. After centrifugation (12 000 *g*, 10 min), the supernatant was collected and the pellet suspended in 5 mL of ultra-pure water. Incubation and centrifugation were repeated, whereafter the supernatants were combined. Nitrate, nitrite and ammonium were quantified spectrophotometrically using a Skalar autoanalyser (Skalar Analytical, Breda, the Netherlands) as described by Strickland & Parsons (1972). Extracts were diluted 40- to 200-fold for analysis. Measurements were calibrated with nitrate, nitrite or ammonium solutions from 0.5 to 10 $\mu$M.

### Statistical analysis

Each nitrate load was analysed from three separate culture trays, and the analyses were performed simultaneously in the glasshouse. Data were normally distributed, and significant differences at $P = 0.05$ between treatments for infected *S. bicolor* or *S. hermonthica* were calculated using the Tuquey test (SigmaStat, SPSS Inc., Chicago, IL, USA). For total Chl, Chl *a* fluorescence and $O_2$ exchange analyses, data are the mean ± confidence interval ($n = 12$ for *S. bicolor*, $n = 20$ for *S. hermonthica*, $P = 0.05$, Student's *t*-test). The other analyses were performed on the DM from all expanded leaves of each plant selected, and data are mean ± confidence interval ($n = 10$ for both *S. bicolor* and *S. hermonthica*, $P = 0.05$, Student's *t*-test).

## RESULTS

### Nitrate-related changes in plant development

Nitrate was supplied to *S. hermonthica*-free roots of *S. bicolor* growing in the sand-bed when the first-emerged parasites were 3 wae (Fig. 1a). The latter are called M parasites because they acquire autonomy in C assimilation


**Figure 1.** Impact of various nitrate supplies on *Striga hermonthica* development. (a) Photograph of *S. hermonthica* and infected *Sorghum bicolor* plants before the nitrate supply. Immature (I) parasites are about 1 wae old. Parasites are mature (M) following 3 wae. For the treatments, nitrate is supplied when the first-emerged parasites are 3 wae old. Photographs (b–d) were taken 3 d following the last supply of nitrate. (b) No symptom reflecting nitrate-related toxicity is observed for the I parasites when nitrate is supplied at 100 mg N per *S. bicolor*. (c) Nitrate toxicity is observed for only the I parasites when cultures are fed with 200 mg N per *S. bicolor*. (d) Nitrate toxicity is evident for both I and M parasites when cultures are supplied with 1500 mg N per *S. bicolor*. (e) Nitrate-related benefit on leaf expansion is evident for the well-expanded leaves of the M parasites. Leaves are collected 2 weeks following the last supply of $KNO_3$. The upper nitrate load of 1500 mg N per *S. bicolor* is lethal for the M parasites (see Fig. 1d).

 

from photosynthesis (Pageau *et al*. 1998). Seeing that emergence is not synchronized in pots, the nitrate load affected simultaneously some M and younger parasites [immature (I)] including subterranean and newly emerged parasites (1 wae old).

Following the low nitrate supplies of 25 and 100 mg N per *S. bicolor*, some parasites still emerged above the ground and no symptom reflecting nitrate-related toxicity was observed on emerged *S. hermonthica*, regardless of their age (Fig. 1b). By contrast, the higher nitrate loads of 200, 500 and 1500 mg N per *S. bicolor* prevented emergence and led rapidly to leaf necrosis and death of I parasites that emerged when nitrate was supplied (Fig. 1c). On the other hand, the photosynthetically M (3 wae) parasites displayed similar toxicity symptoms when nitrate was supplied following only the highest supply of N tested, 1500 mg N per *S. bicolor* (Fig. 1d). Up to 500 mg N per *S. bicolor*, the nitrate supply influenced the *S. hermonthica* phenotype, and promoted a gradual leaf growth (Fig. 1e) and thickening of the stems. Furthermore, the mature leaves of the M *S. hermonthica* became rough, embossed and easily broken when the parasites were fertilized with 500 mg N per *S. bicolor*.

No symptom of toxicity was observed in infected *S. bicolor*, regardless of the nitrate load applied. Rather, the vigour and the pigmentation of the host plants both increased.

## Nitrate-related changes in C assimilation in infected *S. bicolor* and M parasite

### *Impact on total Chl content and photochemical efficiency*

In response to increased nitrate supplies of up to 500 mg N per *S. bicolor*, the Chl content increased gradually in the host which consequently displayed a pigmentation about threefold that of water-sustained plants when fertilized with 500 mg N per *S. bicolor* (Fig. 2a). By contrast, the leaf Chl content did not change significantly in *S. hermonthica* following N fertilization, and Chl trait was clearly reduced when nitrate was supplied at 1500 mg N per *S. bicolor*. Clearly, this is related to senescence. The infected *S. bicolor* did not respond so dramatically to this high N load, and the Chl level remained twice above that of infected plants sustained only with water.

$F_v/F_m$ was calculated from dark-adapted leaves, which then reflected the PSII state. In water-sustained cultures, $F_v/F_m$ was close to 0.8 in the host and in the M parasite, attesting that PSII was normally efficient in

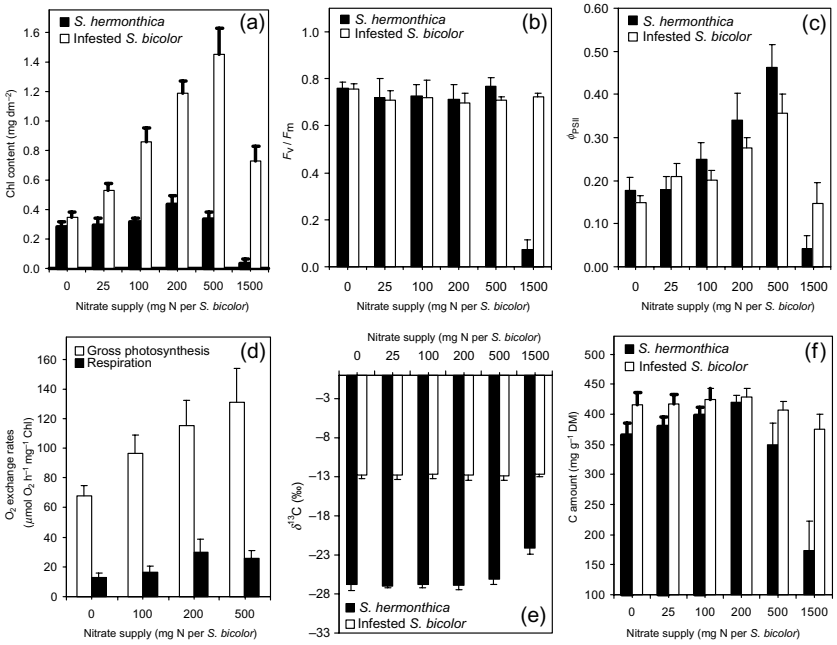


**Figure 2.** Effect of various nitrate supplies on different parameters reflecting C assimilation of well-expanded leaves of infected *Sorghum bicolor* and mature (M) parasites (7 wae old). Nitrate is supplied at 4 and 5 wae. (a–c) Impact on Chl content, $F_v/F_m$ and PSII photochemical efficiency in light-adapted state ($\Phi_{PSII}$), respectively. Values are the mean ± confidence interval ($n = 12$ for *S. bicolor*, $n = 20$ for *Striga hermonthica*, $P = 0.05$, Student's *t*-test). Chl *a* fluorescence was analysed using a pulse-modulated fluorescence monitoring system. $\Phi_{PSII}$ was determined under an actinic light intensity (293 $\mu$mol $m^{-2}$ $s^{-1}$ PFD) close to environment illumination in the glasshouse (300 $\mu$mol $m^{-2}$ $s^{-1}$ PFD). (d) Impact on $O_2$ exchange rates of *S. hermonthica* plants. Rates were measured polarographically in 3-morpholinopropanesulfonic acid (MOPS) buffer containing saturating $HCO_3^-$ concentration. Values are the mean ± confidence interval ($n = 12$ for *S. bicolor*, $n = 20$ for *S. hermonthica*, $P = 0.05$, Student's *t*-test). (e) Impact on the C isotopic composition ($\delta^{13}C$). Measurements were performed by isotopic ratio mass spectrometry. Values are the mean ± confidence interval ($n = 10$, $P = 0.05$, Student's *t*-test). (f) Impact on total carbon. Amounts were measured by elemental analysis. Values are the mean ± confidence interval ($n = 10$, $P = 0.05$, Student's *t*-test).

 

both plant species (Fig. 2b). N treatments did not affect significantly $F_v/F_m$ in both plants, except the senescence-inducing load of 1500 mg N per *S. bicolor* for *S. hermonthica*.

$\Phi_{PSII}$ was measured from light-adapted plants, and depended on both the PSII state and the intensity of the reducing processes in chloroplasts that control $NADP^+$ regeneration. When the plants were adapted to a PFD of 293 $\mu$mol $m^{-2}$ $s^{-1}$ that was close to the environment light of the glasshouse cultures (300 $\mu$mol $m^{-2}$ $s^{-1}$), the $\Phi_{PSII}$ did not change significantly in both plants when fertilized with 25 and 100 mg N per *S. bicolor* (Fig. 2c). Higher nitrate loads, including 200 and 500 mg N per *S. bicolor* significantly promoted photochemical yield in both the host and the M parasite. Such nitrate supplies also improved *qP* (data not shown). Related to senescence, the high nitrate load of 1500 mg N per *S. bicolor* reduced strongly the $\Phi_{PSII}$ and *qP* values in *S. hermonthica* (Fig. 2c, data not shown for *qP*). Similarly, the $\Phi_{PSII}$ and *qP* values decreased significantly in the infected *S. bicolor* in comparison to plants fertilized with 500 mg N per *S. bicolor*. Consequently, the values are close to their basal level in the infected *S. bicolor* sustained only with water.

### *Nitrate-induced changes in $O_2$ exchange rates, C isotopic composition and total C*

Like $F_v/F_m$, the gross photosynthesis rate reflects PSII activity. Gross photosynthesis increased significantly in the M *S. hermonthica* fertilized with 200 and 500 mg N per *S. bicolor* in comparison to water-sustained parasites (Fig. 2d). Similarly, respiration was intensified significantly following these nitrate supplies. Nevertheless, the increase in nitrate load from 200 to 500 mg N per *S. bicolor* did not change significantly gross photosynthesis and respiration rates.

The C isotopic composition ($\delta^{13}C$ value) of *S. hermonthica* was affected by the nitrate-induced changes in gross photosynthesis. Nevertheless, nitrate loads of up to 500 mg N per *S. bicolor* did not affect the $\delta^{13}C$ value, which remained close to –27‰ (Fig. 2e), a typical value for M *S. hermonthica* (Pageau *et al.* 1998). Similarly, the $\delta^{13}C$ value was stable in the infected *S. bicolor*, and was close to –13‰, a typical value for $C_4$ plants (O'Leary 1992). Clearly, nitrate loads of up to 500 mg N per *S. bicolor* did not change significantly the isotopic composition of either plant, indicating that under these conditions the proportion of autotrophic C in *S. hermonthica* leaves remained at about 85%. However, in senescing *S. hermonthica* that received a nitrate load of 1500 mg N per *S. bicolor*, the $\delta^{13}C$ value was enhanced significantly and the deduced proportion of autotrophic C in leaves dropped to 58%.

All the nitrate loads tested did not affect total C in the infected *S. bicolor* (Fig. 2f). Similarly, the total C amount was stable in *S. hermonthica* leaves when a nitrate load of up to 200 mg N per *S. bicolor* was supplied; however, the higher nitrate loads tested induced a significant decrease in C level.

## Nitrate-related changes in N assimilation and in balance with C assimilation in infected *S. bicolor* and M *S. hermonthica*

### *Nitrate-induced changes in total N, N:C ratio and N organic compound contents*

From 200 mg N per *S. bicolor*, fertilization influenced significantly the total N amount and the N:C ratio of *S. bicolor* leaves that increased gradually with nitrate loads (Fig. 3a & b). Nevertheless, N enrichment was markedly higher in the M *S. hermonthica* leaves (Fig. 3a) where the N:C ratio rose spectacularly (Fig. 3b), resulting in a concomitant N status improvement and a decrease in C level (Fig. 2f).

Protein content was positively correlated in the M parasite leaves with N loads applied up to 500 mg N per *S. bicolor* ($R = 0.966$, linear regression, power of performed test ($\alpha = 0.05$) > 0.8 (Fig. 3c). Furthermore, a strong accumulation of FAA, especially asparagine, was clearly seen in *S. hermonthica* when nitrate was applied at and above 200 mg N per *S. bicolor* (Fig. 3d). By contrast, only the two

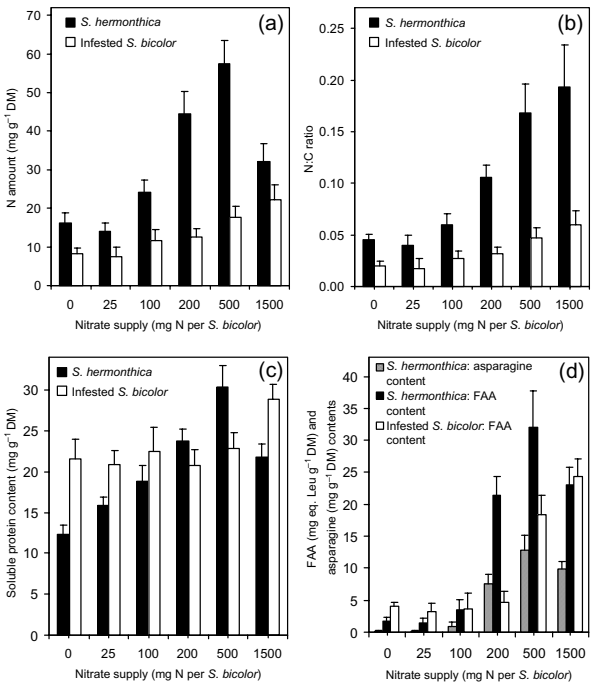


**Figure 3.** Effect of various nitrate supplies on different parameters reflecting N assimilation of well-expanded leaves of infected *Sorghum bicolor* and mature (M) parasites (7 wae old). Nitrate is supplied at 4 and 5 wae. Values are the mean ± confidence interval ($n = 10$, $P = 0.05$, Student's *t*-test). (a & b) Impact on total N and N:C ratio, respectively. Measurements were made by elemental analysis. (c & d) Impact on total soluble proteins and FAA contents, respectively. Asparagine was quantified only in *Striga hermonthica* leaves (d).

 

higher nitrate loads promoted soluble protein and FAA accumulation in the infected *S. bicolor*.

### *Nitrate-induced changes in nitrate, nitrite and ammonium contents*

Up to 200 mg N per *S. bicolor*, the nitrate content was low in the M parasite (Fig. 4a). On the other hand, the nitrate content increased dramatically in response to higher nitrate loads, reaching 14% of the DM following the senescence-inducing nitrate supply of 1500 mg N per *S. bicolor*. Simultaneously, nitrate supplies of 200 mg N per *S. bicolor* led to nitrite and ammonium accumulation (Fig. 4b & c), and ammonium and asparagine contents were shown to be positively correlated [Fig. 4d, $R = 0.988$, linear regression, power of performed test ($\alpha = 0.05$) > 0.8].

Nitrate accumulation is considerably less marked in the infected *S. bicolor* when fertilized (Fig. 4a). Thus, nitrate accumulated following only the higher nitrate load of 1500 mg N per *S. bicolor*. Even then, nitrate represents only 4% of the DM. Similarly, the ammonium content increased significantly when only high nitrate loads were applied (500 and 1500 mg per *S. bicolor*); nevertheless, the extent of accumulation is threefold lower than in *S. hermonthica* (Fig. 4c). Furthermore, unlike in *S. hermonthica*, high nitrate loads did not induce nitrite accumulation in the infected *S. bicolor* (Fig. 4b).

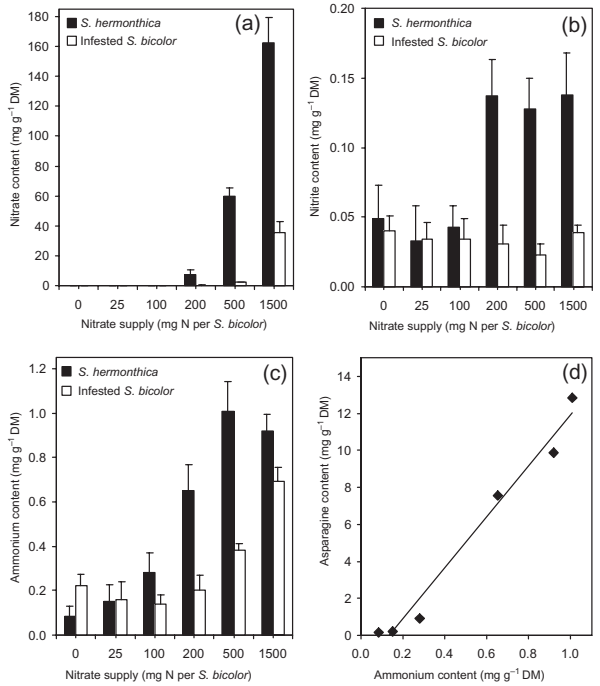


**Figure 4.** Effect of various nitrate supplies on inorganic N accumulation in well-expanded leaves of infected *Sorghum bicolor* and mature (M) parasites (7 wae old). Nitrate is supplied at 4 and 5 wae. Values are the mean ± confidence interval ($n = 10$, $P = 0.05$, Student's *t*-test). (a–c) Impact of nitrate, nitrite and ammonium contents, respectively. (d) Correlation between *Striga hermonthica* leaf ammonium and asparagine contents.

## DISCUSSION

### Nitrate supply improves photochemistry and N assimilation in the infected *S. bicolor*

The photochemical yield is improved gradually in the host by increasing nitrate loads for up to 500 mg N per *S. bicolor* (Fig. 2c). Even if $CO_2$ assimilation was not determined specifically in this study, given the linear relationship between $\Phi_{PSII}$ and $CO_2$ assimilation that exists currently in plants (Genty *et al*. 1989) and the results reported by Cechin & Press (1993b) and Press & Cechin (1994) that showed an improved photosynthetic performance in infected *S. bicolor* when fertilized, we can assume here that nitrate loads of up to 500 mg N per *S. bicolor* improve C assimilation in the infected *S. bicolor*, which is clearly more efficient than in *S. hermonthica* in balancing C and N assimilation under these N regimes (Fig. 3b).

The benefit on photochemistry is lost for the infected *S. bicolor* when fertilized with 1500 mg N per *S. bicolor* (Fig. 2c). When compared to *S. bicolor* fertilized with 500 mg N per *S. bicolor*, other parameters including marked loss in Chl (Fig. 2a), and nitrate and ammonium accumulation (Fig. 4a & c), all indicate that the maximum benefit for nitrate fertilization is situated below 1500 mg N per *S. bicolor*. Nevertheless, plant vigour is enhanced in comparison to water-sustained plants, and no symptom related to nitrate toxicity is observed. Although C assimilation decreases (Fig. 2f), N incorporation into FAA and soluble proteins is still intensified (Fig. 3c & d), suggesting that reducing power is diverted mainly to assimilate excess N.

### Nitrate supply improves photochemistry and asparagine accumulation in M *S. hermonthica*

The benefit of N fertilization on host productivity could also be explained by the additional effect of nitrate directly on *S. hermonthica*. Indeed, the nitrate-related toxicity for the underground parasites (Cechin & Press 1993a; Eplee *et al*. 1994; Press & Cechin 1994) is confirmed in the present study by the inhibition of emergence at and above nitrate loads of 200 mg N per *S. bicolor* (Fig. 1b). Moreover, a high susceptibility is maintained during the slow growth-rate period after emergence, corresponding to the first wae (Fig. 1c). The impact of nitrate supply on C and N metabolism of I *S. hermonthica*, including underground and 1 wae plants, is not clear from the present study and thus deserves further investigation.

By marked contrast, nitrate loads of up to at least 500 mg N per *S. bicolor* improve the productivity of the M *S. hermonthica* plants (3 wae) that consequently display both enhanced C and N assimilatory capacities. This finding is compatible with the patterns of C isotopic composition, photosynthetic capacity, NR activity and expression of *ShAS* gene that were described previously in *S. hermonthica* during its life cycle (Press *et al*. 1987a; Press, Tuohy & Stewart 1987b; Press, Graves & Stewart 1990; Igbinnosa & Thalouarn 1996; Pageau *et al*. 1998, 2003; Sim-

 

ier *et al*. 2005). Autonomy for C, which is shown to be already high in water-sustained parasites, does not change significantly by nitrate supplies of up to 500 mg N per *S. bicolor* (Fig. 2e). Nevertheless, these nitrate supplies have a positive effect on the photochemical capacity of the whole *S. hermonthica*, as a result of concomitant increases in leaf expansion (Fig. 1e), photochemical yield (Fig. 2c) and PSII activity (Fig. 2d). Consequently, photosynthesis can support an increase in plant development when the M parasites are supplied with nitrate loads of up to 500 mg N per *S. bicolor*.

Following high N regimes, free nitrate represents the bulk of the leaf N in *S. hermonthica* (Fig. 4a). Nitrate influx from the host appears to be uncontrolled following fertilization, leading to a dramatic imbalance between C and N economy (Fig. 3a & b). This apparent poor or uncontrolled N accumulation may be partly because of the direct connection of the haustorial tracheid cells of *S. hermonthica* to the conducting xylem vessels of *S. bicolor* roots, which do not facilitate the regulation of water and solute transfer from the host (Dörr 1997). Nevertheless, N supplies of up to 500 mg N per *S. bicolor* in the present study support intensification of N assimilation, as shown by the strong accumulation of organic N compounds, notably in asparagine (Fig. 3c & d). The finding strengthens the proposed central role of asparagine in N management of this plant species (Pageau *et al*. 2003). Asparagine biosynthesis in plants is generally considered to be mediated by AS (Ireland & Lea 1999), which is predicted to be important in some plants in stocking N temporarily in response to N excess and in alleviating ammonium toxicity (Brouquisse, Gaudillère & Raymond 1998; Baldet *et al*. 2002; Carvahlo *et al*. 2003; Harrison *et al*. 2003; Herrera-Rodríguez, Maldonado & Pérez-Vicente 2004; Wong *et al*. 2004). In *S. hermonthica*, *ShAS* was shown to be involved in the diurnal production of asparagine in mature leaves, and was linked to ammonium accumulation in C-starved calluses (Pageau *et al*. 2003; Simier *et al*. 2005). In addition, the present study demonstrates that asparagine content is positively correlated with ammonium content in leaves (Fig. 4d). Consequently, it is reasonable to postulate that asparagine synthesis is crucially involved in alleviating ammonium accumulation in the M parasites. Nevertheless, it is evident that asparagine production is insufficient to dissipate ammonium accumulation under high nitrate loads, including 1500 mg N per *S. bicolor*, in the present study (Fig. 4c). Furthermore, intensification of N assimilation in *S. hermonthica* when fertilized with 500 mg N per *S. bicolor* is shown to be at the expense of C assimilation (Fig. 2f), and this marked the threshold of tolerance for nitrate in M parasites.

## Threshold of tolerance for nitrate in M *S. hermonthica*

Following high nitrate supplies, nitrate is accumulated dramatically in *S. hermonthica*. Even under tolerant conditions, when M *S. hermonthica* plants were supplied with nitrate at 500 mg N per *S. bicolor*, the nitrate pool represents 6% of the leaf DM (Fig. 4a), thus contributing strongly to osmoregulation. Such a spectacular nitrate accumulation is reported in leafy vegetables that utilize nitrate as osmoticum (Chen *et al*. 2004). Given that nitrate is an important osmotic anion for leaf cell expansion in plants (Salsac *et al*. 1987; Raab & Terry 1994), the close relationship between the increase in leaf expansion (Fig. 1e) and in nitrate accumulation (Fig. 4a) in *S. hermonthica* can be understood easily.

The extent of N reduction, hence of ammonium production, will depend on the level of NR activity. Although NR is induced in *S. hermonthica* shoots by N fertilizers, the subsequent NR activity is relatively low in comparison with the NR activity in non-parasitic plants (Tuohy, Smith & Stewart 1986; Igbinnosa & Thalouarn 1996). In the present study, the NR capacity is clearly surpassed following the nitrate load of 500 mg N per *S. bicolor*, as attested by the strong accumulation of nitrate (Fig. 4a). Nevertheless, the M *S. hermonthica* plants benefit from the nitrate supplies as long as the nitrate accumulation and the amounts of toxic compounds, including ammonium and mainly nitrite (Fig. 4b & c) can be tolerated by metabolism. In these experiments, the threshold of tolerance is surpassed by applying a nitrate load of 1500 mg N per *S. bicolor* (Fig. 1d).

## Opportuneness and efficiency of N fertilizers in the field

In conclusion, *S. hermonthica* displays a high sensitivity to nitrate during the early period of its life cycle, but this is reduced significantly following 3 wae. This explains why the *S. hermonthica* seedlings that nonetheless succeed in developing in the field following fertilization can profit from the N enrichment of the soil, thus reducing the efficiency of N fertilizers as a control measure. Nevertheless, the tolerance of M parasites can be surpassed by some high nitrate regimes that are tolerated by the infected plants. In addition, the threshold of nitrate tolerance is shown to be higher in un-infected *S. bicolor* (data not shown), seeing that that benefit on photochemistry is still clear in the single plants when fertilized with 1500 mg N per *S. bicolor*. Simultaneously, nitrate and ammonium did not accumulate in these plants. Consequently, high N regimes shall be considered as an efficient measure to control *S. hermonthica* in the field. However, the environmental and ecological problems that may be induced cannot be omitted, thus strongly reducing the interest of such a treatment. In the present study where the N content taken up by the host roots is easily controlled in the culture trays, some lower N loads (here, 200 mg N per *S. bicolor*) are efficient in preventing emergence and in promoting *S. bicolor* productivity. However, the real amount of N that is available for the plants is not easy to estimate in the field, when both leaching and soil mineralization occur. The breeding of *Sorghum* cultivars that display both resistance (Haussmann *et al*. 2004) and increased capacity in nitrate absorption must be considered to improve the

 

efficiency against *S. hermonthica* of environment-acceptable N regimes.

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