

# Nitrate reductase activity (NRA) of cultivated scrophulariacean root parasites

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Three commonly occurring root parasites (*Alectra vogelii* Benth., *Alectra orobanchoides* Benth., *Striga asiatica* (L.) Kuntze) as well as their hosts were cultivated and their NRA determined. Nitrate reductase activity, albeit extremely low, could be induced with nitrate application. It was concluded, however, that these parasites still had to obtain organic nitrogenous compounds from their hosts.

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Die NRA van gekweekte wortelparasiete (*Alectra vogelii* Benth., *Alectra orobanchoides* Benth., *Striga asiatica* (L.) Kuntze) sowel as hul gashere, is bepaal. Nitraatreduktase-aktiwiteit, alhoewel baie laag, is deur byvoeging van nitraat geïnduseer. Die gevolgtrekking was egter dat hierdie parasiete steeds organiese stikstofverbindinge van hul gashere moes verkry.

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## Introduction

In a previous paper Hunter & Visser (1985) concluded that the transfer of organic nitrogenous compounds from the host into the parasite apparently constitutes a major contribution to the nitrogen of the latter. This conclusion was supported by the fact that no *in vivo* NRA could be demonstrated in the root parasites, except for *Alectra vogelii* Benth. and *Striga asiatica* (L.) Kuntze. It was therefore argued that root parasites might have partly lost their ability to reduce nitrate, in the course of evolutionary adaptation to the parasitic habit. The apparent absence of NRA (Hunter & Visser 1986) might, on the other hand, be due to the fact that only a negligible amount of nitrate enters the parasite. In view of the inducible nature of nitrate reductase (NR), this situation could lead to the ostensible absence of NRA. An attempt to stimulate the synthesis of NR in these parasites therefore seemed necessary, to ascertain whether the observed low NRA was due to the fact that it was not induced or not synthesized at all.

## Materials and Methods

Three root parasites, *Alectra vogelii* Benth., *Alectra orobanchoides* Benth., and *Striga asiatica* (L.) Kuntze were grown in pots in sandy soil on their respective hosts *Vigna unguiculata* L., *Helianthus annuus* L., and *Zea mays* L. Pots were kept in a growth room at 28/21°C, light intensity of 6 000 lux, and photoperiod of 16 h. The host plants investigated were infested by either a few or large numbers of parasites. The growth medium was supplemented with KNO<sub>3</sub> in an attempt to stimulate or increase enzyme synthesis. Those hosts, infested by only a few parasites, were irrigated diurnally, alternately with Hoagland's nutrient solution (double strength KNO<sub>3</sub> = 315 mg N dm<sup>-3</sup>) (Hewitt 1952) and water, for the duration of the investigation. The heavily parasitized hosts were only irrigated in this manner for seven days prior to harvesting. Initially, these plants were irrigated with water only, since it was reported that a high nitrogen application (in the form of KNO<sub>3</sub>) suppressed the development of the parasites (Yaduraju, Hosmani & Prabhakara Setty 1979; Nel 1984, unpublished).

*Striga asiatica* was also grown on *Zea mays* in complete darkness in tubes as described by Visser, Dörr & Kollmann (1977), with one modification. Instead of filling the tube with vermiculite or cotton wool, the bottom end was simply placed in a beaker containing the appropriate nutrient solution. The composition and application of nutrient solution was as described for the lightly infested hosts.

*In vivo* NRA was determined in the leaves of the parasites and hosts as reported previously (Hunter & Visser 1986).

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Control treatments included H<sub>2</sub>O instead of KNO<sub>3</sub> in the infiltration and incubation media.

The NRA is expressed as nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup> after NRA's of the control treatments were subtracted.

## Results and Discussion

The *in vivo* NRA of the leaves of the root parasites, cultivated on their hosts, are shown in Table 1. The results are discussed with reference to the NRA found under field conditions.

### Parasites

From the results it is evident that the root parasites are able to reduce nitrate, although to a lesser extent than the parasitized or the unparasitized hosts. The NR activities range from 0,00 (*A. orobanchoides* on the heavily parasitized *H. annuus*) to 278,00 nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup> for *S. asiatica* parasitizing *Z. mays*.

The fact that the NRA of *A. vogelii* differs when parasitizing two different hosts, could be explained by various factors affecting NRA in higher plants. Such factors are genetic composition, plant species, and available nitrate (Beevers & Hageman 1969; Aparicio-Tejo & Sánchez-Díaz 1982). However, with reference to Hunter & Visser (1986) (Table 2) *A. vogelii*, parasitizing *V. unguiculata*, clearly shows an increase in NRA from 0,35 to 2,28 nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup>, upon stimulation with nitrate.

A light infestation of *H. annuus* resulted in a NRA in *A. orobanchoides* of 11,00 nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup>. When the host was heavily infested, no NRA could be demonstrated for the parasite. It is therefore possible that the root system of the host was disrupted by the penetration of the haustoria of the parasite and therefore became less effective in absorbing nitrate.

The NRA of *S. asiatica* significantly increased upon nitrate application (from 172,00 to 278,00 nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup>). This finding is inconsistent with that of Stewart, Nour, McQueen & Shah (1984) who failed to find any increase in activity following nitrate application to excised shoots as well as intact plants of *S. asiatica* and *S. hermonthica* (Del.) Benth. Death of the parasite occurred instead. The NRA found in the present investigation was also slightly higher than the *in vivo* and *in vitro* activity of 200,00 nmol NO<sub>2</sub><sup>-</sup> gfw<sup>-1</sup> h<sup>-1</sup> reported by Stewart *et al.* (1984) and Shah, Tuohy, King & Stewart (1984) respectively.

When *S. asiatica* was cultured in darkness, no NRA could be demonstrated, although the culture medium was supplemented with nitrate. However, when harvested after three months, the parasites were normally developed. It therefore seems that *S. asiatica* can support itself completely on the host plant. This is in agreement with the findings of Rogers & Nelson (1962) who concluded that sufficient organic nitrogenous compounds had to be translocated from host to parasite.

### Hosts

Except for *V. unguiculata* the parasitic infestations had a definite harmful effect on the NRA of their hosts. Suppression of the NRA of the infested hosts amounted to 41,57–77,95%. In contrast to the increased NRA of the parasites *A. vogelii* and *S. asiatica*, the NRA of the heavily infested *V. unguiculata* and *Z. mays* decreased with nitrate application. The NRA of the heavily infested *H. annuus* being parasitized by *A. orobanchoides*, however, increased. It therefore would seem possible that the parasite might obtain inorganic nitrogen from the host.

**Table 1** *In vivo* NRA of the leaves of parasites and hosts under controlled conditions

| Parasite species<br>Host species   | Unparasitized<br>hosts | Infestation | NRA (nmol NO <sub>2</sub> <sup>-</sup> gfw <sup>-1</sup> h <sup>-1</sup> ) |  | % Suppression<br>of NRA <sup>c</sup> |
|------------------------------------|------------------------|-------------|--|--|--------------------------------------|
|                                    |                        |             | Field conditions<br>(Low N) <sup>a</sup>                                   | Controlled conditions<br>(High N) <sup>b</sup> |                                      |
| <i>A. vogelii</i>                  |                        |             | ND   | 9,22 ± 4,31                                    |                                      |
| <i>H. annuus</i>                   |                        | Light       | ND   | 393,00 ± 98,30                                 | 46,46                                |
| <i>H. annuus</i>                   |                        |             | ND   | 734,00 ± 48,10                                 |                                      |
| <i>A. vogelii</i>                  |                        |             | 0,35 ± 0,00  | 2,28 ± 0,74                                    |                                      |
| <i>V. unguiculata</i>              |                        | Heavy       | 575,00 ± 24,70   | 301,00 ± 25,50                                 |                                      |
| <i>V. unguiculata</i>              |                        |             | ND   | 71,20 ± 6,36                                   |                                      |
| <i>A. orobanchoides</i>            |                        |             | ND   | 11,00 ± 5,50                                   |                                      |
| <i>H. annuus</i>                   |                        | Light       | ND   | 624,00 ± 350,00                                | 41,57                                |
| <i>H. annuus</i>                   |                        |             | ND   | 1068,00 ± 100,00                               |                                      |
| <i>A. orobanchoides</i>            |                        |             | 0,00   | 0,00   |                                      |
| <i>H. annuus</i>                   |                        | Heavy       | 293,00 ± 90,90   | 372,00 ± 31,10                                 | 77,95                                |
| <i>H. annuus</i>                   |                        |             | ND   | 1687,00 ± 211,00                               |                                      |
| <i>S. asiatica</i>                 |                        |             | 172,00 ± 58,00   | 278,00 ± 2,12                                  |                                      |
| <i>Z. mays</i>                     |                        | Heavy       | 788,00 ± 26,20   | 440,00 ± 2,12                                  | 41,57                                |
| <i>Z. mays</i>                     |                        |             | ND   | 753,00 ± 38,20                                 |                                      |
| <i>S. asiatica</i><br>(dark grown) |                        |             | ND   | 0,00   |                                      |
| <i>Z. mays</i>                     |                        | Heavy       | ND   | 245,00 ± 13,40                                 | 43,29                                |
| <i>Z. mays</i>                     |                        |             | ND   | 432,00 ± 43,13                                 |                                      |

<sup>a</sup>Hunter & Visser (1986)

<sup>b</sup>Mean and standard deviation of three replicates

<sup>c</sup>NRA of parasitized host, expressed as % of that of the unparasitized host.

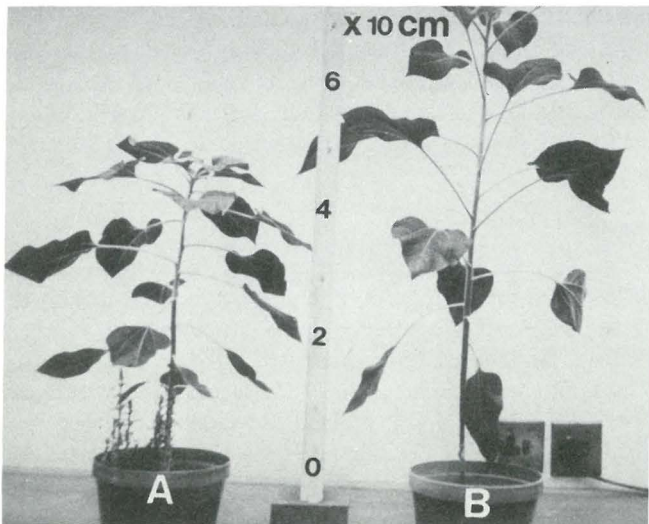
% Suppression = 100 - % Decrease

ND = Not determined.



According to Beevers & Hageman (1969) the potential to induce the NR enzyme is related to the capacity for protein synthesis. Increasing maturation (senescence) was associated with a decreased NR inducibility. This aspect might be a reason for the lowered NRA found in the parasitized hosts, because, in general, parasitic attack is associated with early senescence of the host plant.

In *H. annuus* and *Z. mays* a decreased production or depletion of organic nitrogenous compounds could lead to a reduced yield. This could be due to the removal of nitrate by the parasite and a decreased capacity for protein synthesis because of the growth inhibitory effect of parasitism (Figure 1).



**Figure 1** *Helianthus annuus* parasitized by *A. orobanchoides* (A), showing the growth reducing effect of parasitism compared to the unparasitized host (B).

### Conclusions

From this study it seems clear that, although extremely low (in the case of *A. orobanchoides* and *A. vogelii*), the parasitic flowering plants investigated do exhibit NRA. By implication this would mean that they have not altogether lost their ability to reduce nitrate.

The consequence of a parasitic attack was manifested in the decreased NRA of the hosts. Increased and decreased enzyme activity of parasites and hosts respectively, following nitrate application, would seem to suggest a transfer of nitrogenous compounds from the host into the parasite.

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