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Effects of abscisic acid and nitric oxide on trap formation and trapping of nematodes by the fungus *Drechslerella stenobrocha* AS6.1

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ABSTRACT

The *in vitro* effects of abscisic acid (ABA) and nitric oxide (NO) on the nematode-trapping fungus *Drechslerella stenobrocha* AS6.1 were examined. The average number of traps (constricting rings) per colony and the percentage of nematodes (*Caenorhabditis elegans*) trapped were greatly increased by addition of ABA but greatly suppressed by addition of sodium nitroprusside (SNP, an NO donor) to corn meal agar. The suppressive effect of SNP was not negated by addition of an NO synthase competitive inhibitor (l-naphthylacetic acid, L-NNA) or an NO-specific scavenger [2-(4-carboxyphenyl)-4,4, 5,5-tetramethylimidazoline-1-oxyl-3-oxide, cPTIO]. When added without SNP, however, L-NNA and cPTIO caused moderate increases in trap number and trapping. The results indicate that the trap formation and nematode-trapping ability of *D. stenobrocha* were enhanced by ABA but decreased by exogenous NO.

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Introduction

Nematode-trapping fungi play an important role in regulating nematode numbers under natural conditions and are promising biocontrol agents of nematode pests (Barron 1977). Depending on the species, these fungi attack living nematodes by using one or several of the following five kinds of trapping devices: adhesive networks, stalked knobs, adhesive columns, non-constricting rings, and constricting rings (Stirling 1991). In culture, trapping fungi are induced to produce traps by many factors including nematodes, nemin, animal sera and tissue extracts, yeast extract, and valine (Pramer & Kuyama 1963; Balan & Lechevalier 1972; Nordbringhertz 1973, 1977). Trap induction and morphogenesis could also be affected by abscisic acid (ABA) and nitric oxide (NO) because these important signal molecules are often involved in fungal development (Ninnemann & Maier 1996; Singh *et al.* 1997; Wang & Higgins 2005; Herrera-Medina *et al.* 2007).

ABA is a well-known plant hormone involved in abscission, ABA-mediated signalling, and plant response to environmental stress (Seo & Koshiba 2002). In recent years, ABA has also been demonstrated to be involved in plant resistance to pathogens, i.e., ABA acts as a signal that affects jasmine acid biosynthesis and the activation of defenses in *Arabidopsis* (Adie

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et al. 2007). ABA increased the susceptibility of tomato to arbuscular mycorrhizal infection and was necessary for arbuscule formation (Herrera-Medina *et al.* 2007).

NO is an important signalling molecule in both plant and animal cells. In animals, NO is an intra- and intercellular mediator in apoptosis, inflammation, and neurotransmission (Kim *et al.* 2001). In plants, NO functions in disease resistance, responses to abiotic stress, cell death, respiration, senescence, root development, germination, and hormone responses (Mur *et al.* 2006). However, evidence for the presence and role of NO in microorganisms is limited. Studies with the fungi *Neurospora crassa*, *Colletotrichum coccodes*, and *Aspergillus nidulans* suggest that NO may have a regulatory effect in conidiation and germination (Ninnemann & Maier 1996; Chiuchetta & de Castro-Prado 2005; Wang & Higgins 2005). Confocal microscopy indicated that NO influences appressorium formation in the plant-pathogenic fungus *Blumeria graminis* f.sp. *hordei* (Prats *et al.* 2008). Recent research found that NO activated peroxisome proliferator-activated receptor gamma through a p38 MAPK signalling pathway (Ptasinska *et al.* 2007). The MAPK pathway is essential for appressorium formation (Bruno *et al.* 2004). Because NO has been associated with peroxisome activity and because electron-dense peroxisomal-like microbodies occur in the traps but not in other cells of the nematode-trapping fungus *Arthrobotrys oligospora* (Veenhuis *et al.* 1985), NO could affect trap formation of nematode-trapping fungi.

The constricting ring is perhaps the most elaborate of the traps formed by the nematode-trapping fungi. Each of these traps consists of three curved cells that form a ring with an aperture; when a nematode moves into the aperture and contacts the inner surfaces of the three cells, the cells instantly expand around the nematode and trap it (Barron 1977). In the current study, the effects of ABA and NO on trap formation and on the percentage of nematodes trapped by the constricting ring fungus *Drechslerella stenobrocha* were quantified. Because NO may be involved in some plant physiological functions induced by ABA (Zhao *et al.* 2001; Desikan *et al.* 2004), the combined effect of ABA and NO also was investigated.

Materials and methods

Chemicals

ABA, sodium nitroprusside (SNP), and l-naphthylacetic acid (L-NNA) were purchased from Sigma (St. Louis, MO, USA), while 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) was purchased from Molecular Probes (Eugene, OR, USA). SNP is an NO donor, L-NNA is a competitive inhibitor of NOS (nitrous oxide synthase), and cPTIO is an NO-specific scavenger.

Fungus and nematode

The nematode-trapping fungus *Drechslerella stenobrocha* AS6.1 was selected to evaluate the effect of ABA and NO on trap formation and nematode trapping. The fungus was deposited in the Center of General Microorganisms Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China as a single-conidium isolate and was maintained on slants of potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.).

A wild-type strain (N2) of *Caenorhabditis elegans* obtained from the Caenorhabditis Genetics Centre was used as the nematode prey. Strain N2, which was isolated from mushroom compost near Bristol (UK) by L.N. Staniland, is the canonical 'wild-type' *C. elegans* strain and is used in laboratories throughout the world (Muschiol *et al.* 2009). Nematodes were maintained on 9-mm nematode growth medium (NGM) agar plates containing (per litre of pure water): NaCl (3.0 g), agar (17 g), peptone (2.5 g), $CaCl_2$ (111 mg), cholesterol (5 mg in 1 ml ethanol), $MgSO_4$ (247 mg), and K_3PO_4 (3.35 g). *Escherichia coli* OP50 was cultured at 37 °C for 24 h in L-broth containing (per litre of pure water): NaCl (5.0 g), Bacto-yeast (5.0 g), and Bacto-tryptone (10.0 g). *Escherichia coli* OP50 is a non-pathogenic strain routinely used as a food source for *C. elegans*. The NGM agar was surface inoculated with 0.1 ml of a 24-h culture of *E. coli* OP50 and incubated at 37 °C for 24 h to establish confluent growth before transferring the nematodes. Agar plugs (about 5 mm diam.) with nematodes were transferred to the NGM plates and incubated at 25 °C for 3 d. The surface of the NGM agar plates, containing a dense population of living nematodes of all ages, was washed with 3 ml of distilled water, i.e., the plates with water were gently rotated to float off the nematodes (Bichai *et al.* 2009). The nematode concentration was determined by counting the nematodes in a 10-μl suspension at 100× magnification. The concentration was then adjusted to 100 nematodes per 10 μl.

Preparation of the colonies and measurement of trap formation and trapping

The inoculum of *Drechslerella stenobrocha* AS6.1 was prepared by punching plugs (5 mm diam.) from the margin of a colony that had grown for 2 weeks at 25 °C on Petri plates PDA (Difco Laboratories, Detroit, Michigan). Sterile cellophane disks (1 cm diam.) were placed on plates (6.0 cm diam.) containing corn meal agar (CMA; BBL Microbiology Systems, Cockeysville, Maryland; 4 ml of CMA and ten disks per plate). The agar plugs with *D. stenobrocha* were placed on the edge of the cellophane disks (Fig 1). After 5 d at 25 °C, the fungal mycelium had grown over the cellophane disks, and the disks were transferred to CMA plates (three disks per 6.0-cm-diameter plate) supplemented with different chemicals at 100 μM concentration: CMA + SNP; CMA + L-NNA; CMA + cPTIO; CMA + ABA; CMA + SNP + L-NNA; CMA + SNP + cPTIO; CMA + SNP + ABA; CMA + L-NNA + ABA; and CMA alone. A suspension of nematodes (200 in 20 μl) was then added to each cellophane disk.

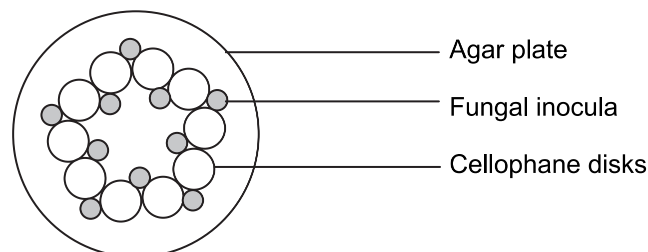

Fig 1 – The position of *D. stenobrocha* inocula (agar plugs) and cellophane disks on CMA plates.

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After 17 h at 25 °C, the numbers of traps, trapped nematodes, and untrapped nematodes were determined at 100× magnification using a Nikon 80i microscope equipped with DIC optics. The percentage of nematodes trapped was calculated.

In a second experiment, the same procedure was followed except that disks were transferred to CMA plates containing ABA at 0 μM, 1 μM, 10 μM, 100 μM, or 1 mM, and only trap formation was quantified.

Statistical analysis

For both experiments, the three replicate plates per treatment were arranged in a randomized block design. Data were subjected to a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $P < 0.05$. SPSS 13.0 software (Statistical Package for the Social Sciences) was used.

Results

With CMA alone in the first experiment (indicated by the first bar in Fig 2A), *Drechslerella stenobrocha* formed about 200 traps per disk (Fig 2A). The number of traps formed was substantially increased with ABA + L-NNA and to a lesser degree by ABA (Fig 2A). Addition of SNP (an exogenous NO source) with or without cPTIO (an NO scavenger), L-NNA (an NO competitor), or ABA suppressed trap formation to zero or near zero. Trap formation was moderately enhanced by L-NNA and cPTIO (Fig 2A).

Trapping (percentage of nematodes trapped) in the first experiment followed a similar pattern as trap formation in that ABA greatly increased trapping, the NO source suppressed trapping (whether or not the NO scavenger, the NO competitor, or ABA were also added), and the NO scavenger and competitor without the NO source moderately increased trapping (Fig 2B).

In the second experiment, the number of traps formed increased as ABA concentration increased from 0 μM to 100 μM and then decreased at the highest ABA concentration of 1 mM (Fig 3).

Discussion

In pre-experiments, the effects of ABA and NO on trap formation by the constricting ring fungus *Drechslerella stenobrocha* AS6.1, the net fungus *Arthrobotrys oligospora* CBS115.81 and the adhesive column fungus *Gamsylella cionopaga* AS3.6776 were quantified (data not shown). And the results showed that 100 μM SNP has widely suppressing effect, but 100 μM ABA has widely enhancing effect on trap formation of nematophagous fungi. However, only *D. stenobrocha* AS6.1 was chosen for this study. One reason for selecting the isolate *D. stenobrocha* AS6.1 for further study was because of its stable morphology which made it feasible to identify and observe traps easily under the microscope. Another reason was its moderate growth rate, which permitted the use of 1 cm diam. cellophane disks to conveniently monitor trap formation and without encountering mycelium ageing. In addition, induction of moderate numbers of traps in *D. stenobrocha* AS6.1 gave statistically significant data, without the need for excessive counting. Therefore *D. stenobrocha* AS6.1 provided an excellent model for these studies.

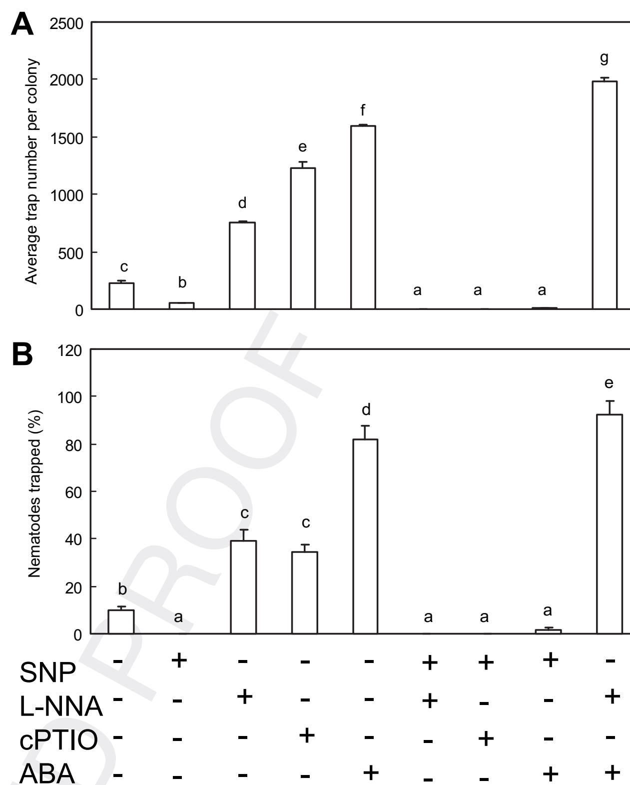

Fig 2 – Trap formation by the nematode-trapping fungus *Drechslerella stenobrocha* and the percentage of nematodes trapped as affected by addition of SNP, L-NNA, cPTIO, and ABA to CMA plates. (A) Trap number per plate. (B) Percentage of nematodes trapped. The control (CMA without addition of chemical) is represented by the first bar on the left. Values are the means + SE of three replicate plates. Bars with different letters are significantly different at $P < 0.05$ (Duncan's multiple range test).

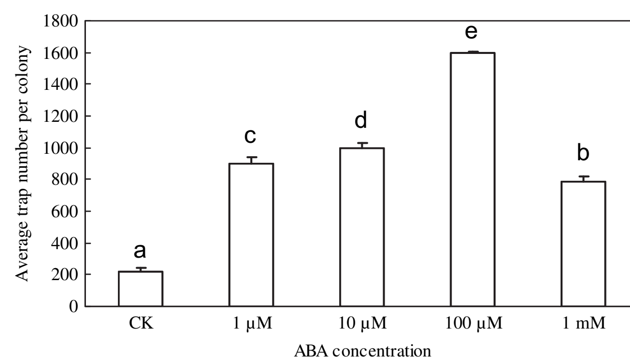

Fig 3 – Trap formation by the nematode-trapping fungus *Drechslerella stenobrocha* as affected by ABA concentration in CMA. Values are the means + SE of three replicate plates. Bars with different letters are significantly different at $P < 0.05$ (Duncan's multiple range test).

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Phytohormones are synthesized not only by plants but also by plant-associated fungi. The best-known example of plant hormones produced by fungi is the gibberellins, named after the ascomycete *Gibberella fujikuroi*, from which they were first isolated. And then biosynthesis of auxins, ethylene, cytokinins and ABA was reported for a variety of plant-associated fungal species (Dörffling *et al.* 1984; Tudzynski & Sharon 2002). ABA is one plant hormone which regulates seed dormancy and induces dehydration tolerance by reducing the stomatal aperture (Leung & Giraudat 1998), and has been shown to be produced by members of different divisions of filamentous fungi which are involved in symbiotic or pathogenic interactions with plants, such as the basidiomycete *Rhizoctonia solani*, the ascomycete *Ceratocystis fimbriata*, and the zygomycete *Rhizopus nigricans* (Dörffling *et al.* 1984; Crocoll *et al.* 1991). However, no detection of ABA production by nematode-trapping fungi has ever been reported.

Effects of exogenous ABA on fungal morphogenesis in plant-pathogenic and arbuscular mycorrhizal fungi have been demonstrated (Singh *et al.* 1997; Al-Masri *et al.* 2002; Fester & Hause 2007; Chaudhary *et al.* 2008). In this paper, we found that ABA also influenced trap formation by the nematode-trapping fungus *D. stenobrocha*. Although *D. stenobrocha* and other nematode-trapping fungi are unlikely to penetrate plant tissue and thereby contact ABA, they are connected to plants in two ways. First, the nematode-trapping fungi capture nematodes that parasitize plant roots. Second and perhaps more importantly, many nematode-trapping fungi are more abundant in the rhizosphere than in root-free soil (Nordbring-Hertz *et al.* 2002). These data suggest an intimate interaction between exogenous ABA and nematode-trapping fungi. Whether the trapping fungi interact with ABA in nature remains to be determined.

NO is an important signalling molecule that participates in many physiological responses in both plant and animal cells (Kim *et al.* 2001; Mur *et al.* 2006). Although there is a little information about the role of NO in microorganisms (Ninnemann & Maier 1996; Chiuchetta & de Castro-Prado 2005; Wang & Higgins 2005), a recent report showed that NO played a key role in appressorium formation by *Blumeria graminis* f.sp. *hordei* (Prats *et al.* 2008). Our findings indicated that exogenous NO suppressed trap formation by *D. stenobrocha* AS6.1 and reducing the NO level will lead to trap formation. These results suggest that NO has regulating effect on trap formation of nematode-trapping fungi by acting as signal molecules for the response.

This research represents an interesting phenomenon to determine the effects of exogenous ABA and NO on trap formation and trapping of nematodes by a fungus *D. stenobrocha* AS6.1. And the molecular mechanism of the ABA- and NO-mediated morphogenesis of nematode-trapping fungi is currently under investigation.

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REFERENCES

Adie BAT, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA, Solano R, 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in *Arabidopsis*. *Plant Cell* **19** (5): 1665–1681.

Al-Masri MI, Ali-Shtayeh MS, Elad Y, Sharon A, Tudzynski P, Barakat R, 2002. Effect of plant growth regulators on white mould (*Sclerotinia sclerotiorum*) on bean and cucumber. *Journal of Phytopathology-Phytopathologische Zeitschrift* **150** (8–9): 481–487. Q3

Balan J, Lechevalier HA, 1972. The predaceous fungus *Arthrobotrys dactyloides*: induction of trap formation. *Mycologia* **64** (4): 919–922.

Barron GL, 1977. *The Nematode-destroying Fungi*. Canadian Biological Publications, Guelph, Ontario, Canada.

Bichai F, Barbeau B, Payment P, 2009. Protection against UV disinfection of *E. coli* bacteria and *B. subtilis* spores ingested by *C. elegans* nematodes. *Water Research* **43** (14): 3397–3406.

Bruno KS, Tenjo F, Li L, Hamer JE, Xu JR, 2004. Cellular localization and role of kinase activity of PMK1 in *Magnaporthe grisea*. *Eukaryotic Cell* **3** (6): 1525–1532.

Chaudhary V, Kapoor R, Bhatnagar AK, 2008. Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. *Applied Soil Ecology* **40** (1): 174–181.

Chiuchetta SJR, de Castro-Prado MAA, 2005. Genotoxic evaluation of sodium nitroprusside in *Aspergillus nidulans*. *Genetics and Molecular Biology* **28**: 798–803.

Crocoll C, Kettner J, Dörffling K, 1991. Abscisic acid in saprophytic and parasitic species of fungi. *Phytochemistry* **30**: 1059–1060.

Dörffling K, Petersen W, Sprecher E, Urbasch I, Hanssen H-P, 1984. Abscisic acid in phytopathogenic fungi of the genera *Botrytis*, *Ceratocystis*, *Fusarium*, and *Rhizoctonia*. *Z. Naturforsch* **39**: 683–684.

Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ, 2004. ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. *Journal of Experimental Botany* **55** (395): 205–212.

Fester T, Hause B, 2007. Drought and symbiosis – why is abscisic acid necessary for arbuscular mycorrhiza? *The New Phytologist* **175** (3): 383–386.

Herrera-Medina MJ, Steinkellner S, Vierheilig H, Bote JAO, Garrido JMG, 2007. Abscisic acid determines arbuscule development and functionality in the tomato arbuscular mycorrhiza. *New Phytologist* **175**: 554–564.

Kim PKM, Zamora R, Petrosko P, Billiar TR, 2001. The regulatory role of nitric oxide in apoptosis. *International Immunopharmacology* **1** (8): 1421–1441.

Leung J, Giraudat J, 1998. Abscisic acid signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 199–222.

Mur LAJ, Carver TLW, Prats E, 2006. NO way to live; the various roles of nitric oxide in plant-pathogen interactions. *Journal of Experimental Botany* **57** (3): 489–505.

Muschiol D, Schroeder F, Traunspurger W, 2009. Life cycle and population growth rate of *Caenorhabditis elegans* studied by a new method. *BMC Ecology* **9**: 14.

Ninnemann H, Maier J, 1996. Indications for the occurrence of nitric oxide synthases in fungi and plants and the involvement in photoconidiation of *Neurospora crassa*. *Photochemistry and Photobiology* **64**: 393–398.

Nordbring-Hertz B, Jansson HB, Tunlid A, 2002. Nematophagous fungi. In: *Encyclopedia of Life Sciences*, pp. 1–10.

Nordbringhertz B, 1973. Peptide-induced morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora*. *Physiologia Plantarum* **29** (2): 223–233.

Nordbringhertz B, 1977. Nematode-induced morphogenesis in the predacious fungus *Arthrobotrys oligospora*. *Nematologica* **23**: 443–451.

Please cite this article in press as: Xu L-L et al., Effects of abscisic acid and nitric oxide on trap formation and trapping of nematodes by the fungus *Drechslerella stenobrocha* AS6.1, Fungal Biology (2010), doi:10.1016/j.funbio.2010.10.006

UNCORRECTED PROOF

521 Pramer D, Kuyama S, 1963. Symposium on biochemical bases of
522 morphogenesis on fungi. II. Nemin and nematode-trapping
523 fungi. *Bacteriological Reviews* **27**: 282–292.
524 Prats E, Carver TLW, Mur LAJ, 2008. Pathogen-derived nitric oxide
525 influences formation of the appressorium infection structure
526 in the phytopathogenic fungus *Blumeria graminis*. *Research in*
527 *Microbiology* **159** (6): 476–480.
528 Ptasinska A, Wang S, Zhang J, Wesley RA, Danner RL, 2007. Nitric
529 oxide activation of peroxisome proliferator-activated receptor
530 gamma through a p38 MAPK signaling pathway. *FASEB Journal*
531 **21** (3): 950–961.
532 Seo M, Koshiba T, 2002. Complex regulation of ABA biosynthesis
533 in plants. *Trends in Plant Science* **7** (1): 41–48.
534 Singh PP, Basra AS, Pannu PPS, 1997. Abscisic acid is a potent
535 inhibitor of growth and sporidial formation in *Neovossia indica*
536 cultures: dual mode of action via loss of polyamines and cel-
537 lular turgidity. *Phytoparasitica* **25**: 111–116.
Stirling GR, 1991. *Biological Control of Plant Parasitic Nematodes: progress,* 538
problems and prospects. CAB International, Wallingford, UK (USA). Q4 539
Tudzynski B, Sharon A, 2002. Biosynthesis, biological role and 540
application of fungal hormones. In: Osiewacz HD (ed.), *The* 541
Mycota X: industrial applications. Springer-Verlag, Berlin, 542
Germany, pp. 183–211. 543
Veenhuis M, Nordbring-Hertz B, Harder W, 1985. Development of 544
fate of electron-dense microbodies in trap cells of the nema- 545
tophagous fungus *Arthrobotrys oligospora*. *Antonie Van Leeuwen-* 546
hoek **51** (4): 399–407. 547
Wang J, Higgins VJ, 2005. Nitric oxide has a regulatory effect in the 548
germination of conidia of *Colletotrichum coccodes*. *Fungal Ge-* 549
netics and Biology **42**: 284–292. Q5 550
Zhao ZG, Chen GC, Zhang CL, 2001. Interaction between reactive 551
oxygen species and nitric oxide in drought-induced abscisic 552
acid synthesis in root tips of wheat seedlings. *Australian Jour-* 553
nal of Plant Physiology **28**: 1055–1061. 554

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Please cite this article in press as: Xu L-L et al., Effects of abscisic acid and nitric oxide on trap formation and trapping of nematodes by the fungus *Drechslerella stenobrocha* AS6.1, Fungal Biology (2010), doi:10.1016/j.funbio.2010.10.006