



Fig. 1. Global pattern of stomatal density of contemporary plants, based on published observations organized and plotted according to the Holdridge Life Zone scheme¹⁰. Values are mapped as the mean stomatal density (mm^{-2}) of all observations for a given type of vegetation (D.J. Beerling and T.M. Smith, unpublished).

with high transpiration rates⁸. Without transpiration, leaf temperatures rose by $>20^{\circ}\text{C}$ above ambient, causing severe heat injury. More recently, the climatic range under which this effect operates has been extended by field observations on plants in flood plain environments in warm temperate regions of Japan⁹.

Viewed at the global scale, we show from an extensive compilation of published data that stomatal densities are highest in tropical vegetation and tend to decline in plants distributed towards the polar regions (Fig. 1), suggesting a relationship between the solar radiation flux, air temperature and plant morphology. This pattern, based on observations, is the converse of what might be expected if plants avoided water loss at all costs, as suggested by Tanner. Instead it might reflect resource optimization allowing maximization of plant carbon gain under a given climatic regime. As part of this optimization strategy, stomatal apertures respond to other aspects of the environment besides air temperature and solar energy. Therefore, contrary to the suggestion of Tanner, we certainly would not anticipate plants opening their stomata at noon because this is the time of day when atmospheric humidity is low and temperature is high. Together these conditions create a high leaf-to-air vapour pressure deficit to which stomata are extremely sensitive in nature⁶ – a phenomenon included in our model.

David J. Beerling*

Colin P. Osborne

Dept of Animal and Plant Sciences, University of Sheffield, Sheffield, UK S10 2TN.

*e-mail: d.j.beerling@sheffield.ac.uk

William G. Chaloner

Dept of Geology, Royal Holloway, University of London, Egham, Surrey, UK TW20 OEX.

References

- 1 Beerling, D.J. *et al.* (2001) Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. *Nature* 410, 352–354
- 2 Royer, D.L. *et al.* (2001) Phanerozoic atmospheric CO_2 change: evaluating geochemical and paleobiological approaches. *Earth-Sci. Rev.* 54, 349–392
- 3 Berner, R.A. *et al.* (2000) Isotopic fractionation and atmospheric oxygen: implications for Phanerozoic O_2 evolution. *Science* 287, 1630–1633
- 4 Edwards, D. (1998) Climate signals in Palaeozoic land plants. *Phil. Trans. R. Soc.* 353, 141–157
- 5 Beerling, D.J. and Woodward, F.I. (1997) Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. *Bot. J. Linn. Soc.* 124, 137–153
- 6 Larcher, W. (1995) *Physiological Plant Ecology* (3rd edn), Springer-Verlag
- 7 Grace, J.C. *et al.* (1995) Fluxes of carbon dioxide and water vapour over an undisturbed tropical forest in south-west Amazonia. *Global Change Biol.* 1, 1–12
- 8 Lange, O.L. (1959) Untersuchungen über den Wärmehaushalt und Hitzeresistenz mauretischer Wüsten- und Savannenpflanzen. *Flora* 147, 595–651
- 9 Matsumoto, J. *et al.* (2000) Ecophysiological mechanisms used by *Aster kantoensis*, an endangered species, to withstand high light and heat stress of its gravelly floodplain environment. *Ann. Bot.* 86, 777–785
- 10 Holdridge, L.R. (1967) *Life Zone Ecology*, Tropical Science Centre, Costa Rica

Nitric oxide: a non-traditional regulator of plant growth

Nitric oxide (NO) research is a new exciting field in plant biology. However, in part because of its chemical properties (gas, free radical, highly diffusible and reactive)¹, the results obtained to date are more intriguing than just interesting². The question about its cytotoxic or cytoprotective roles has already been stated³, and the answer probably depends on accurate measurements of NO concentration *in vivo*.

Several studies have reported that NO can regulate processes related to plant growth and development, some of which are summarized in Table 1. A quick glance at the NO-dependent effects⁴ is enough to raise some basic doubts.

(1) Why does it have so many actions? (2) Why do its effects overlap with those of regulators of plant life, such as photoreceptors and hormones? (3) Do exogenous applications of NO reflect physiological situations? Could NO be defined as a novel plant hormone?

The classical concept of a hormone, as defined by animal physiologists, includes three premises⁵: (1) localized site of biosynthesis, (2) transport to target cells spatially separated from the place of synthesis, (3) control of responses through changes in endogenous levels.

However, plant hormones do not fit into these requirements. First, they are not synthesized in specialized structures that are comparable to animal glands. Although young tissues have a higher capacity for hormone synthesis, the generation of hormones in mature organs can increase suddenly as a result of specific programmes of development⁵. This has led to a more lax concept for plant hormones. In the case of NO, it is mainly formed in actively growing tissues such as embryonic axes and cotyledons, and the levels decrease in mature and senescent organs^{6–8}.

Second, although plant hormones appear to be distributed through the plant, all can exert important functions at the site of their biosynthesis. This shows that transport might not be an essential feature of plant hormones. The small size and high diffusion rate of NO

through biological membranes mean that NO fits the premise that hormones are easily transported.

In regard to the third statement, it is the sensitivity of the target cells, rather than the concentration of the plant hormone that defines the magnitude of a response. Because of this concept⁹, some scientists decided to substitute the term 'hormone' with the wider term 'plant growth regulator', in spite of the confusions that this might generate. Accordingly, phytohormones are widely defined as substances synthesized by plants that affect physiological processes at concentrations much lower than nutrients and vitamins (<1 mM, frequently <1 μ M)¹⁰. NO acts at low concentrations (nM to pM) and most of its functions are dependent on its dose⁴. In this context, NO could be considered to be a plant growth regulator (Table 1).

More recently, five criteria, suggested by Elliot Meyerowitz, added the idea that a plant hormone should be transported at least one cell-diameter distance, and that its action should be accomplished by non-covalent binding to a specific receptor, and remain non-covalently bound, and not covalently modified while acting¹¹. Certainly, NO can react directly to many targets by covalent binding, but the *in vivo* chemistry of plant hormones does not completely exclude covalent binding from their mechanisms of action. Moreover, in mammals, NO not only reacts in a direct fashion but also binds to receptors and shoot signaling pathways, sharing features with hormones and neurotransmitters¹. In plants, there is evidence of cross talk between NO, ethylene, indole-3-acetic acid, abscisic acid, gibberellic acid, calmodulin, cGMP, cyclic ADP ribose and calcium (Refs 2,3,12 and references herein).

Overall, much more work needs to be done before assigning the term plant hormone to NO. The endogenous variations of NO during the plant life cycle need to be monitored. Whether NO acts directly or indirectly through changes in the balance of plant hormones also needs to be determined. A mutational analysis of NO-regulated growth in *Arabidopsis* and the gene cloning of mutant phenotypes should be powerful tools to dissect the cross-talk between NO and other chemical messengers. In the meantime, NO will surely go on surprising us with novel functions.

Table 1. Biological activities of nitric oxide in plants^a

Tissue or organ	Physiological action	Species ^b	Optimum concentrations
Seeds	Induction of germination	<i>Paulownia tormentosa</i> , lettuce, California chaparral (<i>Emmenanthe penduliflora</i>)	10 ⁻⁶
	Inhibition of respiration after imbibition	Soybean ⁸	10 ⁻⁶
	Inhibition of aleurone cell death	Barley ^c	10 ⁻⁶
Roots	Elongation	Maize	10 ⁻¹⁰
	Induction of adventitious and lateral root formation	Cucumber ^d , lavender (<i>Lavandula</i> spp.) ^d	10 ⁻⁹ to 10 ⁻⁵
Tuber	Tuberization	Potato ^d	10 ⁻⁶
Hypocotyls	Inhibition of elongation under low-light fluences	Lettuce, <i>Arabidopsis thaliana</i>	10 ⁻⁶
Stems	Inhibition of internode elongation under low-light fluences	Potato	10 ⁻⁶
Leaves	Induction of de-etiolation	Wheat, barley	10 ⁻⁶
	Delay of senescence	Pea	5 × 10 ⁻⁶
	Stomatal closure	Wheat, <i>Vicia faba</i> ¹²	10 ⁻⁶
	Leaf expansion	Pea	5 × 10 ⁻⁶
	Induction of defence responses	<i>Arabidopsis</i> , tobacco	2 × 10 ⁻⁶
	Inhibition of cell death	Potato	10 ⁻⁶

^aIn plants, nitric oxide (NO) is mainly formed in young actively growing tissues. Because of its high diffusion rate across biological membranes NO can act as both an intra- and intercellular messenger. Targets for NO action include: (i) guanylyl cyclase and haem-containing enzymes leading to enzyme activation or inactivation and (ii) the redox status of the cell, leading to modulation of gene expression.

^bWhere no references are given, examples are reviewed in Refs 2 and 4.

^cM.V. Beligni *et al.*, unpublished.

^dL. Lamattina *et al.*, unpublished (US patent No: 6 242 384 B1; 5 June 2001).

Acknowledgements

We thank Gabriela Pagnussat for critically reading the manuscript. L.L. and M.V.B. are Career Member and Fellow, respectively, from Conicet. L.L. is funded by CONICET, Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Universidad Nacional de Mar del Plata (UNMdP) and M.V.B. is funded by the International Foundation for Science (IFS).

María Verónica Beligni

Lorenzo Lamattina*

Instituto de Investigaciones Biológicas (IIB), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CC 1245, 7600 Mar del Plata, Argentina.

*e-mail: lolama@bart.mdp.edu.ar

References:

- 1 Stamler, J.S. (1994) Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* 78, 931–936
- 2 Wendehenne, D. *et al.* (2001) Nitric oxide: comparative synthesis and signaling in animal and plant cells. *Trends Plant Sci.* 6, 177–183
- 3 Beligni, M.V. and Lamattina, L. (1999) Is nitric oxide toxic or protective? *Trends Plant Sci.* 4, 299–300

- 4 Beligni, M.V. and Lamattina, L. (2001) Nitric oxide in plants: the history is just beginning. *Plant Cell Environ.* 24, 267–278
- 5 Davies, P.J. (1995) The plant hormone concept: concentration, sensitivity and transport. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology* (Davies, P.J., ed.), pp. 13–18. Kluwer Academic Press
- 6 Leshem, Y.Y. *et al.* (1998) Evidence for the function of the free radical gas – nitric oxide (NO) – as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiol. Biochem.* 36, 825–833
- 7 Cueto, M. *et al.* (1996) Presence of nitric oxide synthase in roots and nodules of *Lupinus albus*. *FEBS Lett.* 398, 159–164
- 8 Caro, A. and Puntarulo, S. (1999) Nitric oxide generation by soybean embryonic axes. Possible effect on mitochondrial function. *Free Radic. Res.* 31, S205–S212
- 9 Trewaras, A.J. and Malhó, R. (1997) Signal perception and transduction: the origin of the phenotype. *Plant Cell* 9, 1181–1195
- 10 Crozier, A. *et al.* (2000) Biosynthesis of hormones and elicitor molecules. In *Biochemistry and Molecular Biology of Plants* (Buchanan, B.B. *et al.*, eds), pp. 850–1043. American Society of Plant Physiologists, Rockville, MD, USA
- 11 Leyser, H.M. (1998) Plant hormones. *Curr. Biol.* 8, R5–R7
- 12 García-Mata, C. and Lamattina, L. (2001) Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiol.* 126, 1196–1204