



## COMPARING PLANT AND FUNGAL GRAVITROPISM USING IMITATIONAL MODELS BASED ON REITERATIVE COMPUTATION

David Moore\* and the late Alvidas Stočkus\*\*

\*School of Biological Sciences, The University of Manchester, Manchester M13 9PT,  
U.K.

\*\*Institute of Botany, Žaliujėžeru 48, Vilnius 2021, Lithuania

### ABSTRACT

Mathematical models which imitate plant gravitropic responses were used to compare plant and fungal gravitropism with kinetic data from the agarics *Coprinus cinereus* and *Flammulina velutipes*. Similarities were: bending depends on differential growth; growth of the organ is most intensive just behind the apex; gravitropisms exhibit a substantial time delay. Differences were: the agaric stem apex always returns to the vertical (some plant organs show stable plagiogravitropic growth); curvature compensation occurred in *C. cinereus*; *C. cinereus* stems rarely overshoot or oscillated around the vertical although data for *F. velutipes* showed a single overshoot and oscillation. The work focused attention on the need for data on detection-level thresholds, angle-response and acceleration-response relationships in fungi, and the need for detailed observations of gravitropism kinetics in a larger number and wider range of fungi. ©1998 COSPAR. Published by Elsevier Science Ltd.

### INTRODUCTION

Although the exact nature of the gravity perception/response system in plants is still unknown, there are sufficient kinetic data available to enable mathematical modelling to imitate at least the gross response characteristics of plant organs. Some of these models are in principle universal because their conceptual components are not limited to any specific cellular entities. In this work we used such models to compare plants and fungi to compensate for the much less complete picture of gravitropic kinetics which we have for agaric mushrooms. We hope to use the mathematical model to establish how best to proceed with experimental kinetic analyses specifically tailored to creating a kinetic model of gravitropism in fungi. For this, we have used recently-acquired kinetic data from the agarics *Coprinus cinereus* and *Flammulina velutipes* - (Moore, 1991; Kher *et al.*, 1992; Greening *et al.*, 1993; Novak Frazer and Moore, 1993; Hatton and Moore, 1994; Kern, 1994; Kern and Hock, 1994, 1996; Monzer *et al.*, 1994; Haindl and Monzer, 1994; Monzer and Haindl, 1994; Moore *et al.*, 1994; Monzer, 1995; Greening and Moore, 1996; Novak Frazer and Moore, 1996; Moore *et al.*, 1996). We use an approach in which modelling parameters depend on application of abstract concepts rather than specific cell biological processes. Such an approach has the advantage that it can mimic the overall gravitropic response without detailing how the gravitropic response is realised at the cell or tissue level. The resultant model is an abstraction which can be applied to a wider range of subjects (Stočkus, 1992; Stočkus and Moore, 1996).

## NATURE OF THE MODELS

The basic scheme (Stočkus, 1992, 1994a, c, 1996) assumes that apex angle changes as a result of four consecutive stages - susception, perception, transduction and growth response. Susception is the first physical change (sedimentation, floatation, change in pressure or shear) caused by disorientation, though its nature is irrelevant to the model. The perception step converts the physical change into a physiological one (again, of undefined nature). The transduction stage allows characteristics of signalling processes to be incorporated to allow for the (undefined) growth response which generates the apex angle occurring in tissue different from that which perceives the stimulus.

Mathematical representations of each stage appropriately modify the input to the subsequent stage. Random deviation can also be included but is usually omitted for simplicity. The overall mathematical representation is a set of nested functions which incorporate the known (or assumed) characteristics of each stage. For example, the susception function is assumed to be proportional to the acceleration due to gravity. The perception function describes the relationship between apex angle, gravity vector and the polarity of the effective physiological signal and most other features of the models consequently depend on the attributes of the perception function. Other stages only change how the final angle is achieved but without influencing its value. A variety of models have been described (Stočkus, 1992, 1996) incorporating different aspects of the gravitropic response of plant organs. For example, one model (number 2) introduces a 'diagravitropic' component (as the cosine of the tip angle) which attempts to reflect the common observation that roots more often grow downwards at angles of 10 to 30° away from the vertical than they do exactly vertically (Stočkus, 1994b).

After evaluating available data we chose 26 stems of *Coprinus cinereus* from two different experiments which satisfied the modelling criteria. A standard routine of model fitting was then applied which involved averaging apex angles (in degrees from the gravity vector) of each time course data set for use as initial data, adjustments being made using manual input with visual control of the solutions to establish initial approximate parameters. These provided starting points for the rest of the data for individual subjects and a program of minimisation by the sum of squares was run for each data entry. A constrained method of minimisation by sum of squares was programmed using the unconstrained Newton method as a basis, the termination conditions were norm of gradient (0.01) and number of function calls (1000). Derivatives necessary for the Newton method were calculated using a second degree numerical differentiation formula based on Newton interpolation formulae. The parameters obtained, resultant model curves and their deviations from data points were extracted and used for subsequent analysis. The numerical methods used to solve differential equations and initial conditions were described by Stočkus (1992, 1994c). As a rule, 600 steps were used for solving model fits. Solution of differential equations, estimation of initial modelling parameters and fitting the experimental data were accomplished using programs written by Alvidas Stočkus in Borland Pascal 7.

## RESULTS AND DISCUSSION

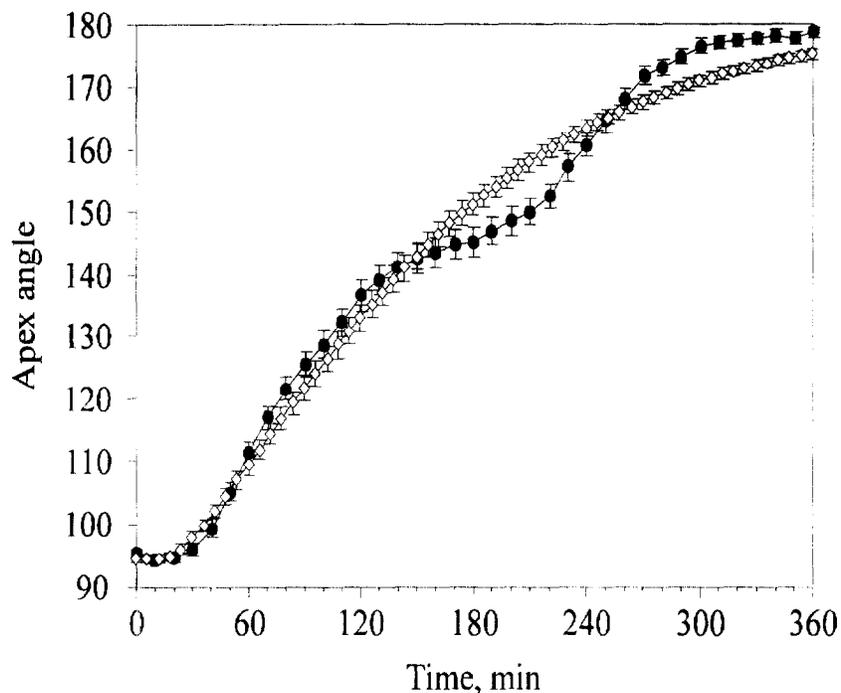
The averaged data of *C. cinereus* tropic response to standard gravitropic stimulation at 90° and the averages of model number 1 fits are shown in Figure 1. Evidently, adequate fits were obtained to the initial stages of the response, but with deviations in the middle and at the end of the response time-courses. The deviations were remarkably consistent revealing: (i) the model predicts a shorter time lag than is normally observed; (ii) the models do not simulate the decreased bending rate which generally occurs at about 2.5 to 3.5 h in *C. cinereus* stems causing the inflected response time

course. Nevertheless, the model fits to *C. cinereus* data were remarkably good (details in Stočkus and Moore, 1996), yielding realistic parameters and strongly indicating that the basic assumptions of the plant models are logically applicable to fungi at least to the extent of providing a good 'first approach' foundation for further detailed analysis.

The adequacy of the fits, particularly over the initial phases of the response, suggest that the basic mechanics of the plant models do indeed apply to fungi. To make a comparison at the cellular level we must find fungal equivalents of the mechanisms which achieve the susception and perception steps in plants. There are no obvious structures responsible for perception of the gravitational impulse in mushroom fruit bodies. Fungal cells generally lack

particulate inclusions which might be candidates as statoliths (discussed in Moore, 1991). Recently, gravity perception in *F. velutipes* was found to be related to the actin cytoskeleton in spindle shaped aggregates around nuclei (Monzer and Haindl, 1994; Monzer, 1995). So a plausible mechanism for gravity perception in agarics (Monzer, 1995) is that nuclei act as statoliths by exerting tension on the actin microfilament system. Similar conclusions have been reached from experiments with *C. cinereus* using cytochalasins to disrupt actin polymerisation (Novak Frazer and Moore, 1996; Moore *et al*, 1996). Thus, there is reason to believe that the physiological basis of gravity perception in mushrooms is similar to that now thought to occur in plants. Clearly, though, more kinetic data are required, particularly about angle-response relationships, acceleration-response relationships and threshold acceleration forces in fungi. Such data would allow improvement of the mathematical model(s) and aid definition of the kinetics of the perception system.

Our analysis also revealed kinetic differences between the two agarics which were compared. The major known difference between *C. cinereus* and *F. velutipes* is a 6-fold difference in the initial rate of response ( $51^\circ \text{ h}^{-1}$  in *C. cinereus*,  $9^\circ \text{ h}^{-1}$  in *F. velutipes*) which results in the apex of the stem of *C. cinereus* reaching the vertical within 3 to 4 h, whilst *F. velutipes* fruit bodies require 12 h to reorient to the vertical. In addition to this, these modelling comparisons showed that *C. cinereus* stems rarely oscillated around the vertical tip angle position, whereas *F. velutipes* showed an exaggerated single oscillation. Again, this was impossible to simulate adequately with existing models. There is evidence in the older mycological literature of even more extreme oscillations. Oscillation around the vertical presumably reflects the speed and accuracy with which the direction of the gravity



**Figure 1.** Averaged data of *C. cinereus* tropic response to gravitropic stimulation (solid symbols) and the averages of all fits to model 1 (open symbols).

vector is sensed and/or the speed and accuracy of the response to such information. The best model fits to the *F. velutipes* observations resulted when the time delay,  $\tau$ , was set at 5 min. and the perception coefficient  $k$  was adjusted to represent a low level of perception. Significantly, one of the suggested differences between *C. cinereus* and *F. velutipes* is that the latter has much less gravity-perceiving tissue (Moore *et al.* 1996). We believe these models do represent a useful starting point, but more detailed information about a wider range of species is urgently required.

We thank the Federation of European Microbiological Societies for a Fellowship which enabled AS to visit Manchester to carry out this work, and the Leverhulme Trust for a Research Grant. Dr Alvidas Stočkus died in a tragic accident in February 1996. His insightful contributions to our developing understanding of fungal gravitational biology will be greatly missed.

## REFERENCES

- Greening, J. P., J. Holden and D. Moore, Distribution of mechanical stress is not involved in regulating stem gravitropism in *Coprinus cinereus*. *Mycol. Res.*, **97**, 1001-1004, (1993).
- Greening, J. P. and D. Moore, Morphometric analysis of cell size patterning involved in gravitropic curvature of the stipe of *Coprinus cinereus*. *Adv. Space Res.*, **17** (6/7), 83-86, (1996).
- Haindl, E. and J. Monzer, Elongation growth and gravitropic curvature in the *Flammulina velutipes* (Agaricales) fruiting body. *Exp. Mycol.*, **18**, 150-158, (1994).
- Hatton, J. P. and D. Moore, Kinetics of stipe gravitropism in the mushroom fungus *Coprinus cinereus* under the conditions of microgravity simulation provided by clinostat treatment. *Microgravity Quart.*, **4**, 101-106, (1994).
- Hock, B., V. D. Kern, J. Monzer and E. Haindl, Influence of gravity on fruiting body development of fungi (Higher basidiomycetes). In *Research Program of the German Spacelab Mission D-2* (ed. P. R. Sahn, M. H. Keller and B. Schiewe), pp. 174-177. DLR: Köln, Germany, (1993).
- Kern, V. D., *Fruchtkörperentwicklung, Gravimorphogenese und Gravitropismus des Basidiomyceten Flammulina velutipes*. Thesis, Technische Universität München, Weihenstephan, Germany, (1994).
- Kern, V. D. and B. Hock, Fungi in space - literature survey on fungi used for space research. *Micrograv. Sci. Technol.*, **6**, 194-206, (1993).
- Kern, V. D. and B. Hock, Gravitropism of fungi - experiments in space. In *Proceedings of the 5th European Symposium on Life Sciences Research in Space* (ed. H. Oser and T. D. Guyenne), pp. 49-60. ESA Publications Division, ESTEC: Noordwijk, The Netherlands, (1994).
- Kern, V. D. and B. Hock, Gravimorphogenesis and ultrastructure of the fungus *Flammulina velutipes* grown in space, on clinostats and under hyper-g conditions. *Adv. Space Res.*, **17** (6/7), 183-186, (1996).
- Kher, K., J. P. Greening, J. P. Hatton, L. Novak-Frazer and D. Moore, Kinetics and mechanics of stem gravitropism in *Coprinus cinereus*. *Mycol. Res.*, **96**, 817-824, (1992).
- Monzer, J., Actin filaments are involved in cellular graviperception of the basidiomycete *Flammulina velutipes*. *European J. Cell Biol.*, **66**, 151-156, (1995).
- Monzer, J. and E. Haindl, Evidence for the involvement of the cytoskeleton and organelles in graviperception of the basidiomycete *Flammulina velutipes*. In *Proceedings of the 5th European Symposium on Life Sciences Research in Space*, (ed. H. Oser and T. D. Guyenne), pp. 89-93. ESA Publications Division, ESTEC: Noordwijk, The Netherlands, (1994).
- Monzer, J., E. Haindl, V. D. Kern and K. Dressel, Gravitropism of the basidiomycete *Flammulina velutipes*: morphological and physiological aspects of the graviresponse. *Exp. Mycol.*, **18**, 7-19, (1994).
- Moore, D., Perception and response to gravity in higher fungi - a critical appraisal. *New Phytol.*, **117**, 3-23, (1991).
- Moore, D., Graviresponses in fungi. *Adv. Space Res.*, **17** (6/7), 73-82, (1996).
- Moore, D., J. P. Greening, J. P. Hatton and L. Novak Frazer, Gravitational biology of mushrooms: a flow-chart approach to characterising processes and mechanisms. *Microgravity Quart.*, **4**, 21-24, (1994).
- Moore, D., B. Hock, J. P. Greening, V. D. Kern, L. Novak Frazer and J. Monzer, Centenary review. Gravimorphogenesis in agarics. *Mycol. Res.*, **100**, 257-273, (1996).
- Novak Frazer, L. and D. Moore, Antagonists and inhibitors of calcium accumulation do not impair gravity perception though they adversely affect the gravitropic responses of *Coprinus cinereus* stipes. *Mycol. Res.*, **97**, 1113-1118, (1993).
- Novak Frazer, L. and D. Moore, The role of calcium accumulation and the cytoskeleton in the perception and response of *Coprinus cinereus* to gravity. *Adv. Space Res.*, **17** (6/7), 87-90, (1996).
- Stočkus, A., Modelling of gravitropic response. 1. A systemic model. *Eksp. biol. (Vilnius)*, **No. 2**, 96-104, (1992).
- Stočkus, A., Basic assumptions and comparison of three gravitropic response models. *Adv. Space Res.*, **14** (8), 145-148, (1994a).
- Stočkus, A., Diagravitropic response in primary plagiogravitropic corn roots. *Env. Exp. Botany*, **34**, 87-94, (1994b).
- Stočkus, A., Modelling of gravitropic response. 2. Competence function. *Biologija (Vilnius)*, **No. 1**, 73-77, (1994c).
- Stočkus, A., Action of gravireceptors: liability hypothesis and model. *Adv. Space Res.*, **17** (6/7), 95-98, (1996).
- Stočkus, A. and D. Moore, Comparison of plant and fungal gravitropic responses using imitational modelling. *Plant, Cell and Environment* **19**, 787-800, (1996).