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Peter Barlow's insights and contributions to the study of tidal gravity variations and ultra-weak light emissions in plants

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- **Background** A brief review is given of Peter W. Barlow's contributions to research on gravity tide-related phenomena in plant biology, or 'selenonastic' effects as he called them, including his early research on root growth. Also, new results are presented here from long-term recordings of spontaneous ultra-weak light emission during germination, reinforcing the relationship between local lunisolar tidal acceleration and seedling growth.
- **Scope** The main ideas and broad relevance of the work by Barlow and his collaborators about the effects of gravity on plants are reviewed, highlighting the necessity of new models to explain the apparent synchronism between root growth and microscale gravity changes 10⁷ times lower than that exerted by the Earth's gravity. The new results, showing for the first time the germination of coffee beans in sequential tests over 2 months, confirm the co-variation between the patterns in ultra-weak light emission and the lunisolar tidal gravity curves for the initial growth phase. For young sprouts (<1 month old), the rhythm of growth as well as variation in light emission exhibit the once a day and twice a day periodic variations, frequency components that are the hallmark of local lunisolar gravimetric tides. Although present, this pattern is less pronounced in coffee beans older than 1 month.
- **Conclusions** The apparent co-variation between ultra-weak light emission and growth pattern in coffee seedlings and the lunisolar gravity cycles corroborate those previously found in seedlings from other species. It is proposed here that such patterns may attenuate with time for older sprouts with slow development. These data suggest that new models considering both intra- and intercellular interactions are needed to explain the putative sensing and reaction of seedlings to the variations in the gravimetric tide. Here, a possible model is presented based on supracellular matrix interconnections.

Keywords: Germination, lunisolar gravity tide, ultra-weak light emission

INTRODUCTION

'Gravity is a uniform background presence during development; it has clearly played a role in shaping the course of plant and animal evolution, and biological constructions are now in harmony with the force that gravity imposes.'

Peter W. Barlow (2007)

Peter W. Barlow's statement constitutes the panoramic window through which the circadian rhythmic behaviour of plants and animals alike can now be scrutinized and meaningfully interpreted. The universality of this vision will be the guiding light of this tribute to a remarkably insightful scholar.

Lunar rhythms are traditionally used by communities all over the world as a tool to assert the best germination and harvest time. This attention to the phase of the Moon is hailed to promote the best yield of the final product, including both quantity and quality of the harvested food or wood (Kollerstrom and Staudenmaier, 2001; Zürcher, 2001).

In his typically forensic and critical way, Barlow explored published data related to such 'Moon-phase' phenomena, and

also helped many groups in exploring new biological data that appeared somehow related to the local gravimetric oscillations, δg , occurring daily as result of the Sun and Moon action (referred to as the lunisolar cycle here) over the Earth's surface gravity. This data mining united and contextualized a wealth of diverse cyclical phenomena such as leaf movements, tree stem diameter and electric potential (EP), stem growth and nutation, root growth, and also spontaneous ultra-weak photon emission (UPE) from sprouts. This contribution will focus on UPEs.

The study of intriguing effects of cyclical lunar phase on plant biology arose only recently amongst Barlow's multiple interests. Among those are ground-breaking forays into the mathematical modelling of plant development presenting novel ideas uniting cell morphology to plant organization and root architecture, or exploring the role of the cytoskeleton in wood cell development in trees (Chaffey, 2017). During his professional employment and also well into retirement, Barlow demonstrated an enduring and enviable ability to explore new territories, on his own, with his own resources, or in collaboration with other scientists embracing a similar fascination for

less trodden yet fundamental aspects of biology. Those who had the good fortune to work and debate with Peter will recognize his boundless generosity and passion for scientific exploration.

To understand the pathway Barlow constructed to advance the study of gravimetric tide effects on plant biology, we need to consider some of his earlier cell and root studies, mainly those pertaining to rhythms, movement and growth.

Barlow discussed how tropic movements in plants would occur in an intricate chart proposed in 1992, trying to elucidate the ways plants would react to external factors, including consideration to gravity (Barlow, 1992). A detailed discussion on plant morphogenesis was then developed in a further substantial article, taking rhythm, periodicity and polarity as the most general properties of living matter:

'[shoot and root growth] depends primarily upon rhythmic switches in the polarity of cell growth. These rhythms coincide with, and may even be dependent upon, unequal potentialities of daughter cells following a quantal mitosis or some *supracellular quantal event* in the apex. (Barlow, 1994; emphasis ours)

What could be such a 'supracellular event'? The question remains open, but we shall get back to it later.

Barlow's specific interest in the oscillatory movements of plants while growing appears in an article of 1994 (Barlow *et al.*, 1994). There, he describes and discusses the minor and major movements of roots, with a special focus on the putative physical information and possible detection threshold involved. In a hallmark twist, he also contends that such movements are the observable, objective expression of the natural presence of external physical drivers – to which humans may be naturally insensitive (Barlow *et al.*, 1994). This vision is reminiscent of what is collectively known as the sensory ecology approach (Dusenberry, 1992), and how the physical organization of nature affects the lives of organisms in space and time (the physical ecology approach).

Along the path of discovery, Barlow contributes further insights and a deep discussion on gravity perception as a sensing capacity that is phylogenetically ancient in plants, and that may probably be exhibited today as a rich functional diversity (Barlow, 1995). There, this important assertion can be found:

'Thus, primitive mechanisms of gravisensing may now co-exist with others which arose independently at later stages of evolution. The discovery of old and new mechanisms together would give the impression that gravisensing is a process with in-built redundancy, although this would not be true if different graviperception systems have distinct roles at particular stages of development.' (Barlow, 1995)

The different gravisensing mechanisms that might exist in a single plant would act towards one common, integrated response, perhaps in redundancy, or act as distinct and complementary mechanisms, each serving the detection of distinct physical quantities. In that work, he also provided a flow-chart for plants and 'lower' organisms that addresses the increase in complexity of the putative detection systems, from those based on statoliths (structures using the migration of dense intracellular calcite or starch granules to sense gravity), which would fit for bacteria and algae, to those based on displacement of the nucleus, fit for fungi,

and finally to amyloplasts (intracellular starch granules within statocytes), as seen in higher plants (Barlow, 1995).

While Barlow was providing advice for experiments to be conducted on the International Space Station (ISS), he discussed the impact of microgravity on plants, pointing to possible developmental disturbances, with insights that may help to resolve the question of the putative 'supracellular' mechanisms:

'Many gravity responses of organisms (their tropisms and taxes) may trace to boundaries, such as the plasma membrane, where mass (acceleration) would be intercepted. The properties of the boundary that follow from interception of acceleration (gravisusception) may then serve as preconditions for further "events", and so on. (...) It would be interesting if, given the range of possibilities in normal development, as exhibited by plasticity, to discover whether gravity supplies any unique preconditions for the accomplishment of a developmental event at either the molecular or morphological levels.' (Barlow, 1998; emphasis ours)

It was also clear to Barlow that root cap dynamics were key to understanding how gravity acts on growth. He later advanced this point further, stating:

'(...) there are intriguing suggestions of some kind of physiological link between the border cells surrounding the cap and mitotic activity in the cap meristem. Open questions concern the structure and functional interrelationships between the root and the cap which surmounts it, and also the means by which the cap transduces the environmental signals that are of critical importance for the growth of the individual roots, and collectively for the shaping of the root system.' (Barlow, 2002)

The rhythmic aspect of plant development in space was developed together with Jacqueline Lück (Barlow and Lück, 2008), where it was stressed that 'the repetitiveness of large-scale branching events, as well as the smaller scale of repeated idiosyncratic cell development, are related to the rigorous clock-like mechanism which governs cell reproduction'. This realization in effect established the need to better understand the temporal (rhythmic) aspects of morphogenesis, a theme that is thereafter omnipresent in Barlow's approach to plant development and its plasticity, and this will be evident later.

Barlow's first study on the relation of leaf movements to the local gravimetric tide appeared in 2008 (Barlow *et al.*, 2008), where he carefully developed a meta-analysis of Klein's data (Klein, 2007). Barlow and collaborators showed that the nastic movement of leaf blades appears synchronous to the local gravimetric changes due to the relative movement of Sun and Moon. Numerous examples, from different species and cultivars, indicated that an increasing tidal force usually depresses the leaf downwards, and that rapid leaf bending movements occurs when there is a local change in the tidal microgravity δg , i.e. when such 'force changed from either a minimum ("low tide") or a maximum ("high tide")' (Barlow *et al.*, 2008). In keeping with Barlow's efforts to spread news concepts and ideas, he suggested a name for gravity tide-related phenomena: 'selenonastic' effects (from the Greek *Selene* = Moon) (Barlow *et al.*, 2008).

The similarity between δg time patterns and the diameter variation of tree stems was later explored with collaborators (Barlow *et al.*, 2010). Exploring published data from seven species of tree at two different locations growing in natural and in controlled conditions, this article proceeded to examine tree stem oscillation patterns in view of local gravity fluctuations. While mindful of the distinction between causation and correlation, this paper concluded that the lunar component of the gravity variation alone could influence stem diameter variation and that, ‘under certain circumstances, additional regulation may come from the geomagnetic flux’ as well (Barlow *et al.*, 2010).

At that point, Barlow felt that solid specific empirical data were needed and, now retired, he secured key collaborations, renewing his efforts to collect crucial data. Importantly, the co-variation with local gravimetric tide was also found for the growth of *Arabidopsis thaliana* roots (Barlow and Fisahn, 2012; Fisahn *et al.*, 2012; Barlow *et al.*, 2013). Using accurate video recording of root tips in controlled conditions, growth velocity was traced in time, and growth was found to be in phase with the δg profile. The data show common periodic components between δg and the rate of root elongation. In that study (Barlow *et al.*, 2013), the irregular natural variations of the solar geomagnetic flux reaching the Earth was also considered, and proposed as a possible additional factor acting over growth cycles. Although geomagnetic storms are quite rare and unpredictable, and difficult to avoid in normal laboratory conditions, they are known to be correlated with Sun and Moon positioning in relation to Earth, akin to the δg tide. In effect, disentangling the lunisolar tidal effects from those relating to variations in the geomagnetic flux is considered quite difficult to determine in standard laboratory conditions (Barlow *et al.*, 2013).

Barlow (2012) developed further the idea of gravity tide as an extrinsic ‘developmental modulator’ of life processes. There, he hypothesized that the adaptive value of the mechanisms at work for gravity sensing are also related to the bio-availability of water – a molecule evidently crucial and ubiquitous for germination and growth. In this work, Barlow mined historical data of bio-EP from trees collected by different groups in the 1940s, 1990s and 2000s. The diverse data available show that the daily EP cycle is co-variant with the δg tide, and that their respective amplitudes are related in a linear way. Monthly time-resolved data clearly exemplified the co-occurrence of EP oscillations and δg during an entire Moon cycle. EP fluctuations were also shown to be proportional to water content in the tree, rather than to transpiration rate. Altogether, a link was proposed between water content and bio-availability, and the gravimetric tide (Barlow, 2012).

The hypothesis that the local gravimetric tide is acting over leaf movements was supported by results of tests run in the ISS (Fisahn *et al.*, 2015) – during its 90-min orbit around the Earth the ISS undergoes two complete tidal cycles and leaves presented cyclical ascent and descent with 45- and 90-min periods, occurring in synchrony and phase congruence with the lunisolar tidal force, even for different illumination conditions.

A review on leaf movements and their relationship with the lunisolar gravitational force appeared in Barlow (2015), including many new plots for different species where leaf changes appears co-variant to δg cycles. The increasingly abundant supportive data permitted Barlow to develop ever more detailed hypotheses, suggesting once again that:

‘a lunisolar clock, in which the *zeitgeber* is exogenous and independent of metabolism would lie in a category of “primal” biological phenomena that could allow both animal and plant organisms to continue to express rhythmic patterns of behaviour under conditions where light is absent.’ (Barlow, 2015)

The last of his contributions on seedling movement appeared only recently, showing that stem growth, nutation and leaf movement in peppermint (*Mentha piperita* L.) also follow local δg cycles, corroborating the enticing proposition of a gravimetric tidal *zeitgeber* (Zajaczkowska and Barlow, 2017).

How far are we now from understanding the causes and mechanisms of variation of growth in plants, and how complete is our current phenomenological description? Enticingly, another, previously elusive, physical parameter has attracted some attention in the past decade: the UPE occurring in growing seedlings and their relation to δg cycles.

UPE is understood to be a consequence of radiative decay (luminescence) of electronically excited states of molecules which are continuously generated in metabolically active organisms (Cifra and Pospíšil, 2014). The mechanism underlying the generation of excited states and consequent UPE is currently understood to be as follows: metabolism, and its associated oxygen consumption, which takes place mainly in mitochondria, chloroplasts, peroxisomes and endoplasmic reticulum, but also in cell wall-bound oxidases, membrane NADPH oxidases and apoplasts (Møller, 2001; Das and Roychoudhury, 2014; del Río, 2015), leads to the production of reactive oxygen species (ROS). Reactions of ROS with a wide range of lipids, proteins and nucleic acids can produce high-energy intermediate molecules: dioxetanes (Bastos *et al.*, 2017) and tetroxides (Miyamoto *et al.*, 2007). These molecules can decompose to produce either excited species such as triplet excited carbonyl (Bastos *et al.*, 2017) or singlet oxygen (Miyamoto *et al.*, 2007), which can, in turn, directly emit photons or, after incurring some delay, transfer excited state energy to acceptor molecules (Cifra and Pospíšil, 2014). These acceptors can then also emit photons (Cifra and Pospíšil, 2014). This description pertains only to the direct pathway which leads to production of UPE. However, at every step described above, there are many other competing pathways which do not lead to UPE.

The UPE from sprouts during growth was discovered some time ago by Colli *et al.* (1955) and is generally understood as a manifestation of metabolic activity during germination and early seedling growth (Rafieiolhosseini *et al.*, 2016) and has been used as real-time, non-invasive diagnostic probe of vigour (Gallep, 2014). UPE was also found to be related to lipid peroxidation (Havaux *et al.*, 2006) and to ROS activity under flooding stress (Kamal and Komatsu, 2015) in plants.

The first study reporting cycles in spontaneous UPE from seedlings in relation to the local gravimetric tide benefited from generous help and support from Peter Barlow (Moraes *et al.*, 2012). The evidence gathered shows that seedling growth is accompanied by UPE, and that the UPE signal presents temporal variations similar to that of local δg , exhibiting coincident turning points and related periodic components. Analysis of long-term time series of consecutive germination tests over a duration of about 2 months, during which UPEs were recorded for the 2nd and 3rd days of germination of wheat

(*Triticum aestivum* L.), showed also that both UPE intensity and sprout's elongation vary in a similar way during the lunar month. Furthermore, the UPE for a single sunflower seedling also appears to co-vary with the local δg (Gallep, 2014).

Because geolocalization determines the shape of δg variation, experiments were also conducted at two different locations in parallel, e.g. in Neuss, Germany, using local and transported samples, and in Limeira, Brazil, using local samples (Gallep et al., 2013). Testing variations of UPE as a function of different δg input, the data from that study reveal that the UPE of both local samples has profiles with similar periodic components (as assessed using Fourier transforms) to those of the local gravimetric tide (~12.2 and 24.4 h). Of note is the occurrence of many coincident inflection points in time. Interestingly, the UPE profile of the seeds transported from Brazil to Germany presented time profiles with reduced periodicity, lacking coincident inflection points with local gravity tide. In effect, Fourier analysis reveals periodic components distinct from those of the main δg cycles, exhibiting some beat frequencies and additional harmonics (around 6, 15 and 18 h). It must be noted that the days when the seeds were transported by air (São Paulo to Neuss) coincided with a period of strong geomagnetic disturbance related to sunspot activity, a composition of circumstances difficult to replicate that may have influenced seed development.

A second test with transported seeds was run with support of labs in Japan (Hamamatsu city) and Czech Republic (Prague), in September 2012 (Gallep et al., 2014). UPE of wheat germination tests run simultaneously in these two localities and in Limeira, Brazil, all showed co-variation with its local gravimetric profile. Notably, tests conducted in Japan used samples transported from Brazil 1 week earlier and did not show abnormal UPE variation. The same seed stock was brought back to Brazil and measured in parallel and simultaneously to non-transported, resident samples; both presented similar UPE profiles, with regular turning points coincident with those of the local gravimetric variation (Gallep et al., 2014).

A further contribution in this field appeared recently, in collaboration with colleagues at the University of Leiden, Netherlands (Gallep et al., 2017). UPEs were measured from wheat seedlings taken from a stock transported overseas from Brazil to the Netherlands (direct São Paulo to Amsterdam) and back, in July 2014, taken in parallel with those from local stocks. In this case, strong UPE–gravimetric correlations were measured for the local samples, as expected, and for the sample transported from the Netherlands back to Brazil. Similarly to the tests conducted earlier in Neuss, the samples tested in Leiden that had been transported from Limeira lacked the periodicity of the other samples. A linear relation between the local amplitude of δg and of UPE was present for both local and transported samples, indicating that both disturbed (transported) and resident samples do respond more strongly to gravity tide cycles with larger magnitudes. It was also shown that the UPE profiles for seedlings of different species measured in parallel, e.g. wheat–corn and corn–sunflower (*Helianthus annuus*), present turning points consistently coincident with those of the local gravity tide (Gallep et al., 2017).

Here, we present new results of UPE for coffee seedlings, taken during the whole germination period of 2 months. Owing to their slow germination, coffee seedlings enable long-term

recordings to be made, which have revealed the presence of slower variations not found before for sprouts with faster germination: as seedlings mature, the periodic components of the gravimetric tide become gradually less pronounced, unveiling a slower periodicity, with a period of about 4 d.

MATERIALS AND METHODS

Coffee germination

Coffee beans (*Coffea arabica*), selected to discard damaged beans at the Federal University of Lavras (UFLA – MG, Brazil), were put to germinate at 5 February 2016 using standard methods with water-saturated filter paper rolls vertically arranged over plastic containing trays. This stock of seedlings were moved in optimal conditions to University of Campinas/Limeira (SP, Brazil) on 6 February 16 and kept inside germination chambers, in the dark, with controlled constant humidity ($70 \pm 10\%$) and temperature ($32 \pm 2^\circ\text{C}$) for the next 2 months. This is the usual germination time for coffee. Eight filter paper rolls containing 50 seedlings each were maintained through February and March 2016, from where samples were collected every week for the photon-count (PC) tests.

Photon-count tests of seedlings

PC measurements of the spontaneous light emission were run for seedling samples chosen from the stock. Starting on 10 February 2016, and every week thereafter, a sample of seedlings was selected as representative of actual sprout stage and put in a Petri dish, with a 10-cm-diameter filter paper and 10 mL of demineralized water. To obtain a strong light signal, 12 seedling sprouts were positioned on the paper without touching each other. During February PC measurements contained 12 seedlings per run, in March this was reduced to eight seedlings (t1) and then to six seedlings (t2 and t3) per run.

The transfer of seedlings from rolls to dish was done under minimal light exposure to avoid strong delayed luminescence; the dish was then put inside the dark chamber for PC measurements, whereby the sample holder provided temperature control through a regulated thermal bath (set to 32°C) to optimize growth (Gallep, 2014). PC acquisition started just after placing samples in the dark, integrating counts in 10-s time discretization, and recorded continuously throughout the PC test (about 7 d).

Each sample was photographed before and after PC measurements, to assess seedling development in comparison to the main stock.

Data analysis

PC data (counts per 10 s) of each test were smoothed, reducing signal variance by averaging adjacent 100 data points. A second-order polynomial fitting was then used to remove the long-term trend for each test, and the remaining oscillatory profile was again smoothed by adjacent 1000 data points averaging to reveal inflection points and enable comparison with the smooth gravimetric tide profile.

Gravimetric tide calculation

A computer program (ETIDE) was used to calculate the gravimetric tide, which essentially provides an estimate of the local gravitational pull resulting from the combined actions of both Sun and Moon at a chosen location on Earth. ETIDE is based upon the 50 parameters, first used by Longman (1959), for computing the vertical gravimetric component of the lunisolar tidal force. The horizontal component was not included in the present lunisolar tide computations. Briefly, the inputs to ETIDE consist of the latitude, longitude and altitude of the location in question (Limeira, SP, Brazil; 22°33'53"S, 47°24'06"W, 700 m elevation) together with the calendar dates for which estimates are required. The computational output is δg , expressed in μGal ($1g = 9.81 \times 10^8 \mu\text{Gal}$), and represents the increase and decrease of the Earth's gravitational acceleration at any particular location brought about by the combined gravitational forces of the Sun and Moon.

Periodic components

To determine the frequency content of both gravimetric and PC data, periodograms were created using Welch's method (Welch, 1967) with 50 % window overlap, which allows the recovery of low-frequency data. Here, frequency content is given in cycles per day (d^{-1}). Spectra were normalized such that their

total power is equal to 1, facilitating comparison of relative powers at specific frequencies.

RESULTS

The PC data after initial smoothing but prior to detrending are presented in the Supplementary Data, as well as pictures taken from each sequential sample just before and after PC measurement.

The detrended PC data (d-PC) for three tests in February 2016 are presented in Fig. 1, and also for three more tests in March 2016 (Fig. 2). Data are superimposed to the local δg and its first derivative, $\delta g/\delta t$. Due to a momentary technical failure, one test at the beginning of March was lost, constituting the only discontinuity in PC measurements of the 2-month germination test. Test t3 in March (23–26 d, Fig. 2) has its vertical axis (d-PC) divided by 3, to normalize it to other tests, since this last sample exhibited very strong light emission (see PC data in Supplementary Data).

Inflection points of the d-PC curves are highlighted with a pair of vertical arrows, pointing to direct comparison with both time-resolved δg and $\delta g/\delta t$ functions. Numerous, but not all, d-PC inflections are coincident with variations in the local gravitational pull, with most rapid changes in d-PC coinciding with fast inflections in δg , where the rate of change ($\delta g/\delta t$) is maximal.

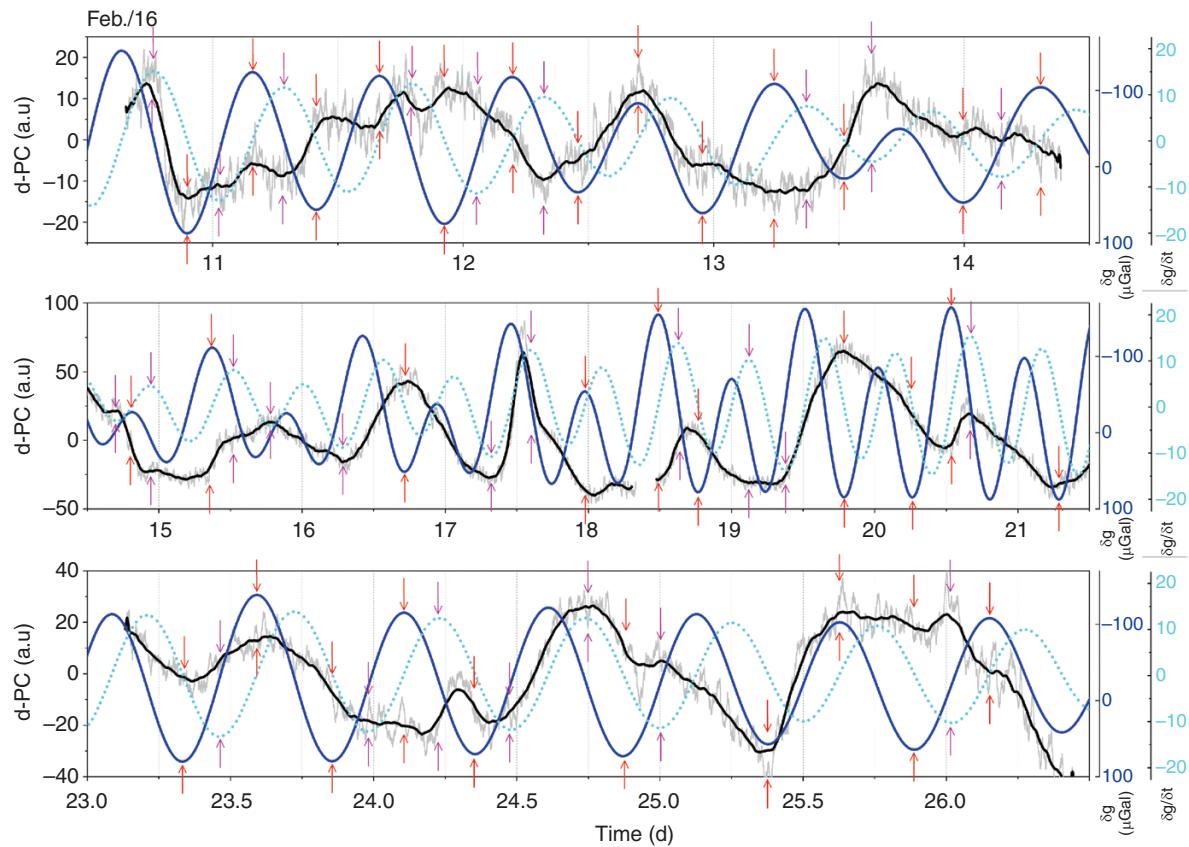


FIG. 1. Detrended photon-count [d-PC, with local smooth of 10^2 (grey line) and 10^3 points (black line)] and local gravimetric tide (δg , blue line) and first derivative ($\delta g/\delta t$, point, light-blue line) data for germination tests of coffee beans in optimal conditions, February 2016. Pairs of vertical arrows (same time) mark significant turning points of δg (red arrows) or to $\delta g/\delta t$ (purple arrows) in coincidence to local/trend changes in d-PC data.

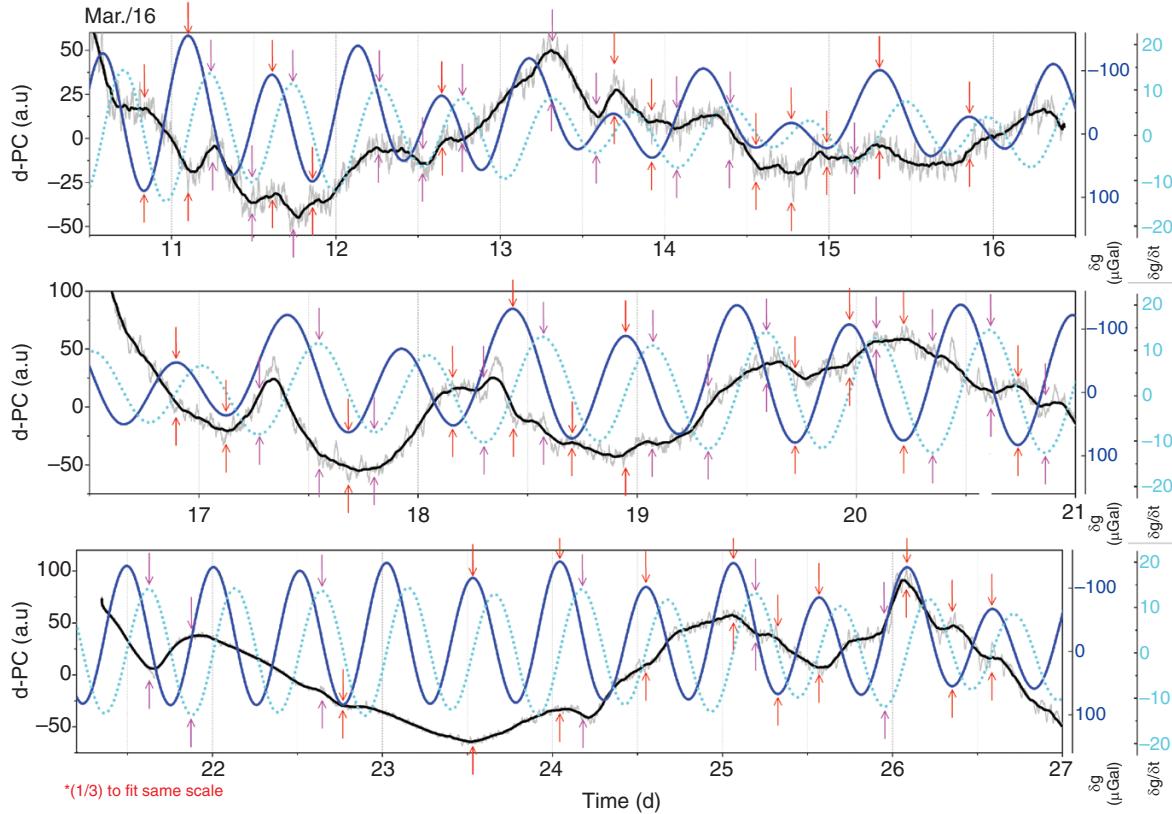


FIG. 2. Detrended photon-count [d-PC, with local smooth of 10^2 (grey line) and 10^3 points (black line)] and local gravimetric tide (δg , blue line) and first derivative ($\delta g/\delta t$, point, light-blue line) data for germination tests of coffee beans in optimal conditions, March 2016. Pairs of vertical arrows (same time) mark significant turning points of δg (red arrows) or to $\delta g/\delta t$ (purple arrows) in coincidence to local/trend changes in d-PC data. For test t3 (23–26 d) the vertical axis (d-PC) is divided by 3, to fit similar scale of other tests.

The long-term temporal organization of the PC data and how it relates to lunisolar cycles can also be depicted in the frequency domain (Fig. 3). The normalized PC periodograms for all six tests, three on each month, reveal the regularity of UPE. When considered along the frequency structure of the δg function, the common periodic components with the photon emissions become apparent (Fig. 3B). In the linear ordinate plots, main trends in amplitude variations are apparent across the dynamic range of the response, while at logarithmic scale, small variations in amplitude are highlighted, revealing the presence of low-power components of the d-PC oscillations.

DISCUSSION

Coffee requires a very long germination period of 2 months under optimal conditions for sprouts to develop both roots and leaves. This is much slower than other species tested previously, such as corn and wheat, whose sprouting takes less than 1 week, corresponding to a quarter of a lunar cycle. In contrast, the development of coffee sprouts spans the passage of two lunar cycles. This means that the same sample of seedlings can be measured continuously along multiple cycles of the semi-diurnal gravimetric oscillations. In addition, long recording times also enable the evaluation of the full range and gravimetric amplitude variations, changing from maximum to minimum four times in 2 months (Figs 1 and 2).

Similarly to previous work on other plant species, initial seedling growth shows numerous coincident inflection points between d-PC and δg profiles, and also coincident long-term trends, with d-PC progressing very similarly to the δg curve. This is exemplified by data for time periods 12.5–13, 17.4–18, 24.5–25 and 25.3–25.6 d in February 2016 (Fig. 1). Yet, this effect tends to diminish as the sprouts get older and the d-PC exhibits a decrease in the amplitude of the periodic components around 1 d^{-1} and 2 d^{-1} (Fig. 3, March). A moderate and progressively waning response is observed around 11.2–11.5, 13–14, 17.2–17.7 and 25.8–26.2 d of March 2016 (Fig. 2).

Another recurrent feature in this set of data is that the coincident inflection pairs usually alternate between δg and its first time derivative, with few exceptions noted mostly for the last test in March. These data suggest that gravimetric tide minima and maxima affect the UPE of seedlings, but also that the rate of change of δg , its velocity, causes variations in photon emissions.

Noteworthy is that the last test (t3, March) exhibited very strong light emissions, despite the presence of only six seedlings, with average photon counts higher than 150 s^{-1} (see Supplementary Data).

This evidence reveals that as the sprouts get older and bigger, photon counts increase, presumably accounting for the mass increase of metabolically active tissue. This is similar to UPE observed in other species (Gallep, 2014). However, the cycle components with frequencies around 1 d^{-1} and 2 d^{-1} ,

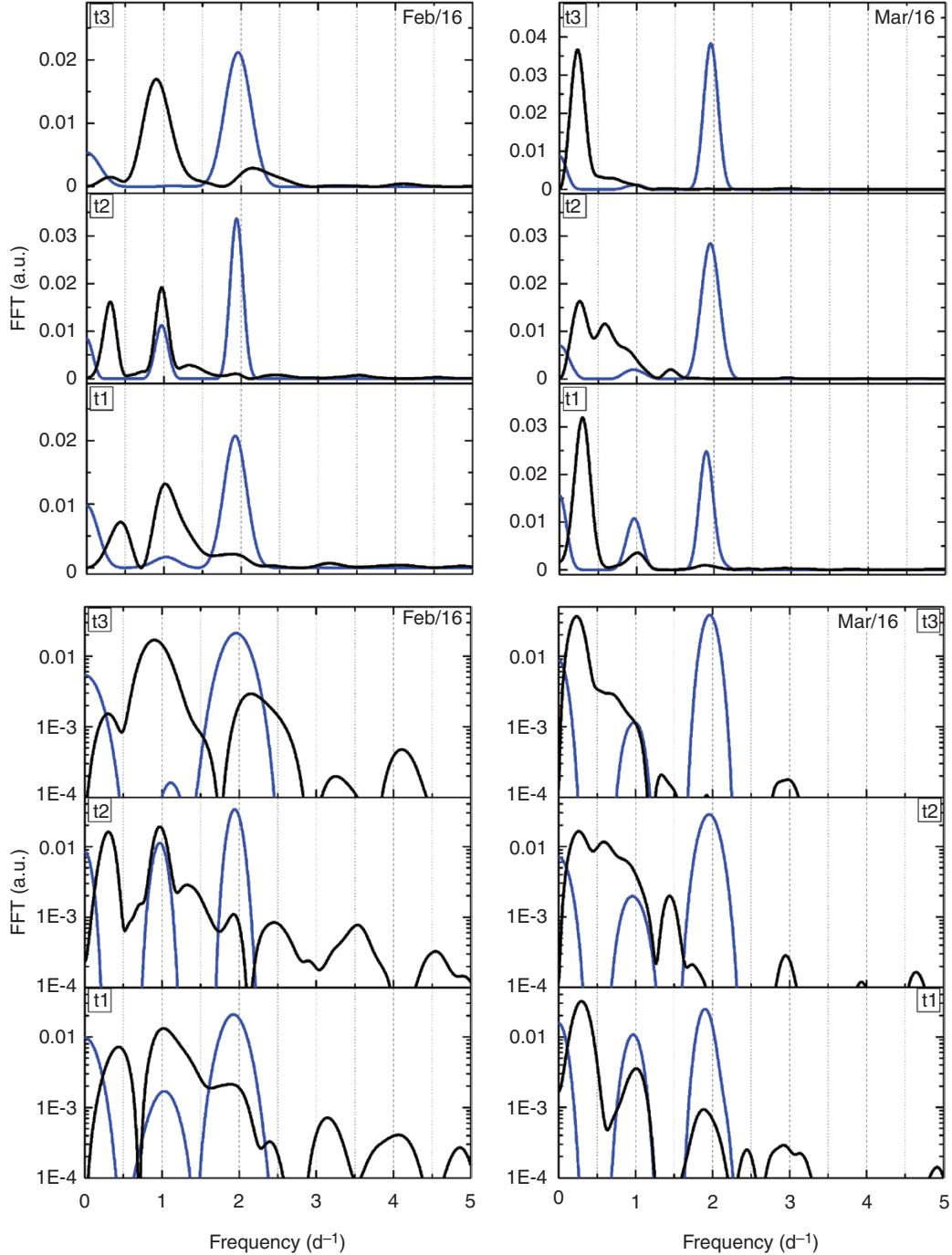


FIG. 3. Periodograms for the d-PC data (black curves) and gravimetric function δg (blue curves), with normalized amplitude, for the following tests: February: t1 (10.5–14.5 d), t2 (14.5–21.5 d) and t3 (23–27 d); March: t1 (10.5–16.5 d), t2 (16.5–21 d) and t3 (21.5–27 d), in linear (upper panels) and logarithmic (lower panels) scales.

that were pronounced at the beginning, fade away at the end of March's tests, when longer period components ($f < 0.51 \text{ d}^{-1}$) contribute to the majority of the oscillatory power (Fig. 3). Components of one cycle per day are always present for the d-PC data, even when the δg component is small around that periodicity (as in t3 February). The main δg periodic component, around 2 d^{-1} , appears also for d-PC data in February, with very small amplitude for t1 March (approx. 10^{-3} , see Fig. 3 log scale).

One possible explanation for the fact that older seedlings showed reduced UPE period components, as compared to younger seedlings, is that, as they have more developed leaves, they would be more sensitive to light. Even if minimal, exposure to light can occur when transferring samples from the growing stock to the PC setup. Such transfer took place once in 1 week. This potential exposure to light could constitute a cumulative input for the older samples, possibly contributing to the very low-frequency UPE periodic components.

One outstanding question pertains to how small changes in local gravity can have an effect on the growth of seedlings. In effect, the amplitudes of variation of δg are estimated to be around 10^6 to 10^7 smaller than the average Earth's acceleration g . How can roots and/or leaves sense microscale variations in gravity, and the forces induced by them? This issue is related, we propose, to the so-called kT paradox whereby, in standard conditions, temperature vibrations at room temperature, or thermal motion, is at least as large as the effects δg can theoretically impart on the plant tissue. Considering the buoyancy intrinsic to cell organelles in their environment, and the estimated δg (approx. 100 μGal), it can be predicted that large bodies are needed (approx. 100 μm radius) for microgravitational pull to overcome thermal motion. This theoretical critical size (and mass) is however greater than the conventionally accepted gravisensing organelle, the amyloplasts. At their largest, amyloplasts located in root cap statocytes have a radius of tens of micrometres (Hinchman and Gordon, 1974). In addition, owing to medium viscosity, the gravity-driven response of amyloplasts requires forces to act steadily for durations in excess of 1000 s. The present evidence reveals response times in the range of tens of seconds, introducing a discrepancy between the dynamics of amyloplast motion, UPE response and the drive by gravimetric tide (see Supplementary Data). In this context, it is worth mentioning that the gravimetric sensing considered here is not atypical of mechanical sensing in general. In effect, across all hearing animals studied, the auditory sensory organs readily sense variations in pressure, called sound by definition, that are at least 10^8 times smaller than the static atmospheric pressure (Robert and Göpfert, 2002).

We contend that a physically plausible mechanism for sensing microgravity forces must involve multiple amyloplasts, or other dense structures. By way of hypothesis, it may be worth considering here the proposition that these are the interactions – or concerted actions – between a collection of amyloplasts that enable, putatively, the sensing of small variations in gravity. This contention is similar to that proposed by Peter Barlow in 1995.

By analogy to active mechanisms in hearing and their role in frequency selectivity and enhanced sensitivity, an active process operating outside thermal equilibrium is hypothesized to be present in the root tip and to be sensitive to small δg perturbations. Tentatively, we posit here that mechanoreceptive molecular mechanisms may not be localized within one cell only, limited to its small collection of amyloplasts, as plant gravitropism is conventionally understood to work (Barlow, 1995). We surmise that ensembles of amyloplasts mechanically connected by cytoskeletal filaments (or other, perhaps yet undescribed, structures) are involved, operating together across several adjacent cells, that is at the supracellular level. Also, speculatively, sensitivity to microscale gravity variation could involve the mesoscopic action of water, since coherently organized and mobile clusters of water could provide electrical–mechanical input to cells in response to conformational or positional changes induced by the gravimetric tide.

This supracellular hypothesis was discussed with Peter Barlow in the past years, and he had many assertive points to claim that this could be possible. Barlow and Chaffey have shown that cytoskeletal elements associated with the cell wall help during cell division, involving myosins, microtubules and microfilaments and acting cooperatively in a supracellular matrix to support growth:

‘(...) Linking the cytoskeleton-mediated long-distance symplasmic transport within the axially oriented sieve tubes/sieve cells with the radial pathway of solutes mediated by the ray cells would create a *super-symplasmic continuum* which would, in turn, permeate the whole tree. The role that this three-dimensional network might have for co-ordination of developmental processes remains to be explored.’ (Chaffey and Barlow, 2002; emphasis ours)

The essential idea emerging from this line of thought and evidence is that intercellular cytoskeletal connections may constitute the substrate for sensing microscale variations in gravity – an assertion that clearly requires to be tested experimentally. Herewith, we propose a conceptual model involving a long-range network of supracellular connections across the root cap (Fig. 4). The organization of this network is overlaid to an original picture of a corn root tip from Barlow (2003). The key proposition resides in the use of the orientation of cellular walls as the guideline for the structured network. By following the continuous lines formed by cell walls, three main types of ‘lanes’ can be distinguished: longitudinal, U-like and transverse. The longitudinal lanes are long, running from proximal to distal and arranged orthogonally to the U-like lanes. U-lanes are located around the proximal root cap edge (Fig. 4), extending laterally to run in parallel to the lateral lanes. Altogether, this network could act as a mechanical strain gauge sensitive in three directions. Mechanical sensitivity is proposed to be provided by the concerted actions of amyloplasts, acting as the inertial elements, and linked molecular components, including microfilaments and motor proteins such as myosins. This proposition is evidently speculative and hopefully can generate the impetus for the development of novel molecular genetics assays, accompanied by biomechanical work to address the molecular composition of intercellular connections, the nanoscale sensitivity of cytoplasmic filaments associated with amyloplasts, and further studies into the susceptibility of root caps to small changes of gravimetric variations.

SUPPLEMENTARY DATA

Supplementary data are available at <https://academic.oup.com/aob> and consist of the following. Fig. S1. Photon-count data (PC, local smooth of 102 points) for series of coffee germination tests, superposed by quadratic fitting (y-axis, dot-dash grey line) to be removed and so explicit oscillations around the main trend, i.e. the detrended photon-count (d-PC) shown in the main text. Fig. S2. Left: threshold force on a single amyloplast needed to overcome thermal noise as a parameter of amyloplast radius and the time over which the deterministic force acts. Right: force exerted on an amyloplast by microgravity fluctuations as a parameter of amyloplast radius and number of amyloplasts. Time series for the PC data for germination tests, including the second-order fit used to detrend signal growth, are presented. Pictures of each sample before and after PC measurements are shown as well as an explanation on calculations for the microgravity force over a cell organelle.

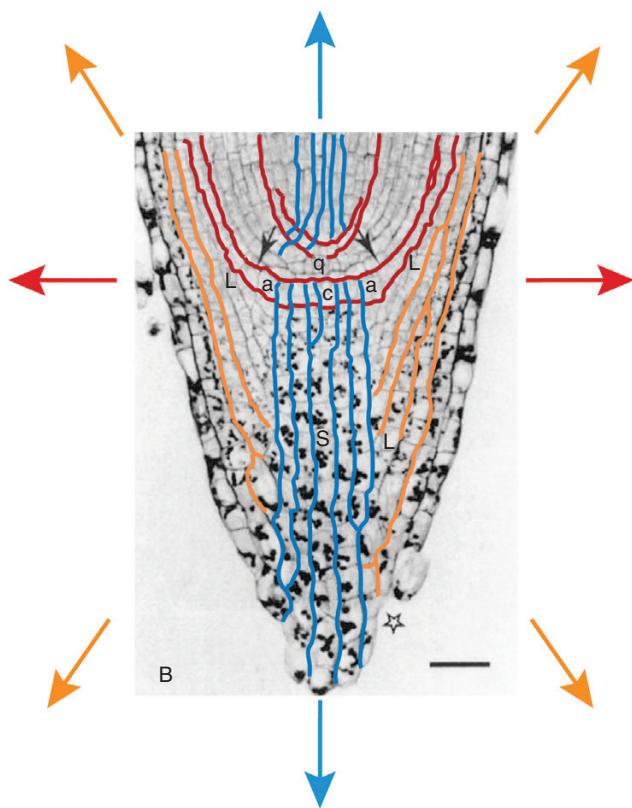


FIG. 4. Illustration for a model of microtubule/microfilament networks in the root cap, highlighting some examples of longitudinal lanes (blue), U-like lanes (red) and lateral, transverse lanes (orange), with respective arrow axis of maximum response (by colour). Drawn over original micrograph of Barlow (2003): root-cap boundary (black arrows), protoderm initials (a), columella initials (c), quiescent centre (q), statenchyma (S) with prominent starch grains and forming part of the group of axially orientated cells which comprise the columella, lateral root cap (L); the star (*) indicates a possible site from which a cell has detached.

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