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STEADY STATE THERMAL CONDITIONS INSIDE PLANT TISSUE CULTURE VESSELS SUBMITTED TO A CONSTANT LEVEL OF IRRADIATION

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URBAN L. and JAFFRIN A. *Steady state thermal conditions inside plant tissue culture vessels submitted to a constant level of irradiation.* BIOTRONICS **19**, 71–81, 1990. A simple mathematical model for quasi-stationary heat and mass transfers inside plant tissue culture vessels was developed and applied to various physical conditions. The validation of the convective coefficients was done with the help of actual temperature measurements performed *in situ* under strictly dry conditions. The extension to water vapor mass transfers inside the vessels was then obtained by a similarity argument. When applied to describe stationary conditions, the model shows that there is ample room for modifications and improvements of the internal micro-climate of the vessels, in particular by increasing the artificial light level, without creating any noticeable thermal stress to the plants. Natural light should however be used with care, owing to its high intensity and the subsequent greenhouse effect in these particularly small size enclosures.

Key words: *in vitro*; micropropagation; radiation level; air temperature; numerical simulation.

INTRODUCTION

The effect of environmental factors has long been emphasized in field and greenhouse cultural methods, and bioclimatology has become a major field of study. However, these questions have not been of great concern in the more recent field of micropropagation, also known as *in-vitro* culture. The reason may be that other parameters were found to be of primary importance, like the composition of the nutrient medium and the proper hormone balance. Furthermore, as the aim of micropropagation is basically different from ordinary plant growth, photosynthesis was not supposed to play a major role in this process. However, more and more works tend to show that this restricted approach of *in-vitro* cultural methods should now be reconsidered.

For example, results of experiments suggest that increased irradiance (*5*, *9*), or modified light spectrum (*4*, *7*, *8*), may influence morphogenesis. Plants placed in micropropagation units are usually submitted to stable artificial physical con-

ditions: a very low irradiation level (often below 10 W/m^2), and constant air temperatures in the range of 20 to 25°C. It is however known that some species present optimum growth conditions which are quite different from such values (*2*, *3*). There are now many evidences that growth taking place during multiplication and acclimatation stages, and survival rate of *in-vitro* propagated plantlets, are enhanced under increased photosynthetic photon flux (PPF) and CO_2 enrichment: plantlets placed in such favorable conditions can in fact grow autotrophically, without needing any more carbone hydrates inside the substrate. This feature could contribute to simplify cultivation methods in the prospect of mass production by reducing pathological risks (*6*).

It is therefore quite appealing to try to act on the environmental factors of *in-vitro* culture vessels, for orienting the micropropagation. It should however be kept in mind that the reduced size of the vessels and their weak thermal inertia make them more vulnerable to thermal agressions. Care should be taken to control the impact of an increased irradiance level on leaf temperature.

As the thermal inertia of small culture vessels is quite small, relatively to potential heat exchanges with the ambience, it is possible to assume, for the time being, all thermal transfers to be quasi-stationary. This makes the computations simpler and the conclusions easier to analyse. A simple model, which treats heat exchanges between the vessel and its environment is presented in the next chapter; then results of actual measurements in usual *in-vitro* conditions are given in chapter 2 and confronted to predictions from the model; finally, in chapter 3, an extrapolation to higher radiation levels is considered.

1. MODEL FOR THE THERMAL BEHAVIOR OF A CULTURE VESSEL

Contrary to usual greenhouses, where radiative gains and heat losses are intense and where the soil has a strong damping effect on the temperature fluctuations, micro-culture vessels involve only light components with small thermal inertia. It is therefore liable to consider such vessels in quasi-stationary states at a given time and to approximate all temperature profiles by linear gradients. When the physical conditions of the environment vary, the vessels are supposed to go from one stationary state to another one, which simplifies the approach.

Geometrical representation of the culture vessel

A typical culture vessel is sketched in Fig. 1. Inside the vessel, the culture medium forms a layer of constant thickness, made of translucid gel with optical and thermal properties close to those of water (Fig. 2). For the sake of simplicity, the model will ignore the presence of the plantlets, which would not account for their mass but rather for their optical absorptivity in the range of photosynthetic wave lengths, and for their ability to exchange water vapor, oxygen and carbon dioxide with the air. Mass exchange measurements are beyond the scope of the present study, so that only a modified irradiation absorptance coefficient for the gel will be introduced in order to simulate the presence of the plantlet.

Culture vessels are usually arranged along two dimensional periodic arrays,

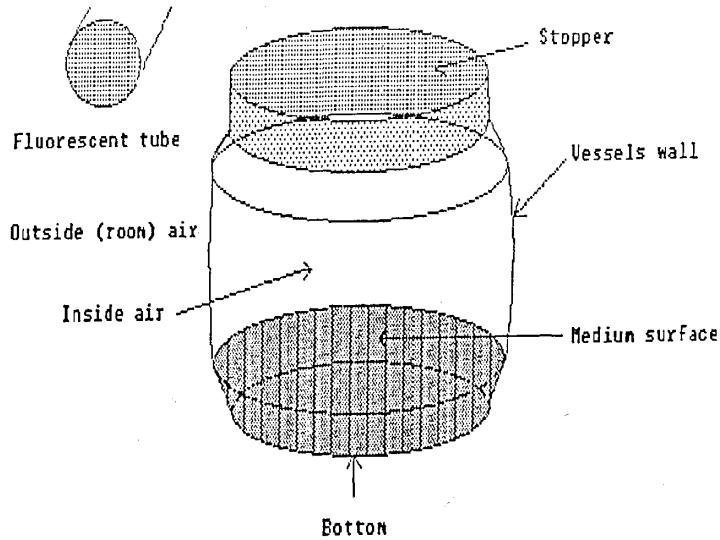

Fig. 1. Typical culture vessel.

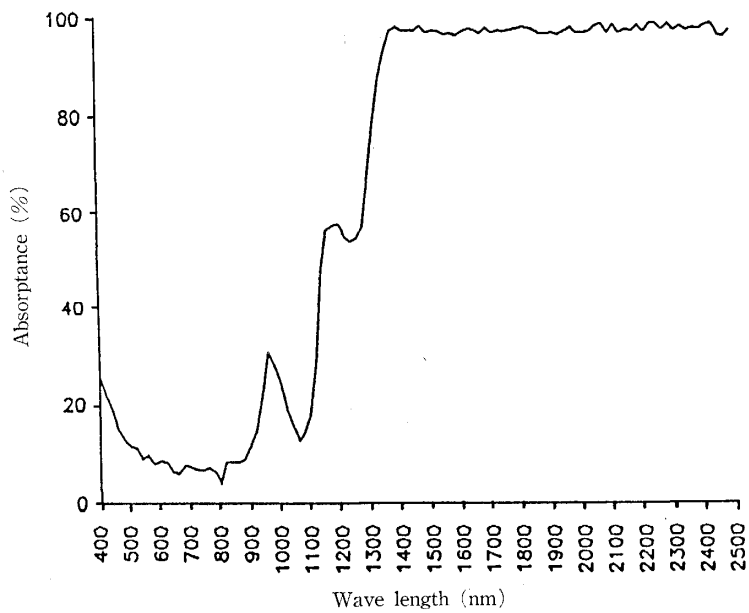

Fig. 2. Absorptance of a 1 cm thick layer of gel medium.

on opaque shelves or thin clays, below racks of discharge bulbs or fluorescent tubes delivering short wave radiations and far infrared.

Classification of heat transfers

The physical environment of a representative vessel includes the presence of similar vessels situated around itself, ambiant air at a controlled temperature and humidity level, and room walls at given temperatures; but, most important are the numerous fluorescent tubes or bulbs, delivering the necessary photosynthetic radiations within a known emission spectrum, which are responsible for a far

infrared contribution that can be estimated from their surface temperature.

For the sake of simplicity, a uniform mean temperature will be assigned to each distinct surface of the vessel (cover, lateral wall and bottom). This means that the small temperature gradients due to a radiative anisotropy and to natural convection effects are neglected. Within this approximation, all heat exchange between adjacent vessels will be omitted. A similar approximation consists in replacing the linear temperature gradients across solid walls or across the culture gel by flat temperature profiles. This does not mean, this time, that heat flows across these walls will be neglected, but it reduces the number of significative parameters in the global description of the system.

External heat transfers include, at first, radiative exchanges between the vessels and their surroundings (walls and sources of light). Such radiative exchanges can be estimated with great accuracy provided sufficient care is taken in computing the angular form factors between opposite surfaces. On the other hand, heat exchanges from convective origin are more difficult to estimate because they involve various patterns of air motion (forced, free or mixed convection depending on detailed temperature gradients on various walls). It will be therefore necessary to justify with experiments the adopted values for the convective coefficients.

1) Radiative exchanges

The usual distinction between short- and longwave radiations is made: the transparent materials used for vessels (glass or polymere) are supposed to stop radiations with wave lengths greater than 2500 nm only and to transmit a major part (90%) of the shortwave radiation emitted by the fluorescent tubes (except for an occasional absorptance in the culture gel medium). In the domain of longwave radiations (thermal radiations), all surfaces are supposed to be grey and opaque (i.e. with equal emissivity ε and absorptivity α, both being independant of the wave length).

The radiative balance between two opposite surfaces S_1 and S_2 at temperatures T_1 and T_2 (in K) writes:

$$\Phi_r = \varepsilon_1 \varepsilon_2 \sigma S_1 F_{12}(T_1^4 - T_2^4)$$

where F_{12} is the form factor (i.e. $1/4\pi$* the average solid angle) through which the surface S_2 is seen from the surface S_1, and σ is the Stephan-Boltzman constant.

It is convenient and traditional to extract from the temperature bracket the term $(T_1 - T_2)$ and to define a mean temperature T_{12} by writing:

$$(T_1^4 - T_2^4) = (T_1 - T_2) 4 T_{12}^3$$

with $$4T_{12}^3 = T_1^3 + T_1^2 T_2 + T_1 T_2^2 + T_2^3$$

Then Φ_r is simply written as:

$$\Phi_r = h_r S_1 (T_2 - T_1)$$

where $$h_r = 4\varepsilon_1 \varepsilon_2 F_{12} \sigma T_{12}^3$$

This will enable to reduce the problem to an apparently linear iterative procedure starting with an initial guessed value for T_{12}. After each step, a new value for T_{12} is calculated for the next iteration, until convergence.

2) Convective exchanges

The general expression for a convective exchange contribution between a surface S and a fluid of thermal conductivity K is given by:

$$\Phi_{cv} = \text{Nu } K\, S/L\, (T_f - T_s)$$
$$= h_{cv} S(T_f - T_s)$$

where T_s is the surface temperature of the solid (which may differ slightly from the average temperature of the flat profile across the solid), and T_f the fluid temperature. The whole complexity of the convection process is contained into the Nusselt number Nu (or the coefficient of convection h_{cv}), which must be deduced from the literature, once the precise nature of the convection taking place around the vessels is known. This is often forced convection when a fan circulates air throughout the shelves; in that case the Nusselt number is usually correlated to the Reynolds number characteristic of the flow under the form:

$$\text{Nu} = Cst\ \text{Re}^a \qquad \text{where Re} = V L/\nu$$

the exponent a being dependant on the regime of the flow (laminar or turbulent), V being the fluid velocity, L a characteristic dimension of the vessel in the direction of V and ν, the kinematic viscosity of the fluid. The constant may differ for the horizontal convection on the covers and for the periodic flow pattern taking place between vertical vessels.

In the absence of forced convection, buoyancy forces may be at the origin of natural air motions (for example plumes formed at the contact of the hot fluorescent tubes or natural convection along the vertical sides of the irradiated vessels); in which cases the Nusselt number is expressed in terms of the Grashof number Gr.

$$\text{Nu} = Cst'\ \text{Gr}^b \qquad \text{where Gr} = g\beta\,\delta T\, L^3/\nu^2$$

the exponent b being also dependant on the regime of the flow, while β is the dilatation coefficient of the fluid, g the constant of gravity, and δT the temperature difference between the wall and the fluid.

It will be necessary, for the precise evaluation of the Nusselt number in the present situation, to determine the regime of the flow by velocity measurements. Then the present geometry of adjacent vertical cylinders must be related to standard configurations for which reliable measurements have been analysed in terms of Nusselt correlations and reported in the literature.

3) Mass transfers

The presence of free water in the culture gel and the biological activity of the plantlet placed in the vessel are responsible for several sorts of mass transfers: water vapor emission (evapotranspiration) and subsequent condensations on the walls of the vessels, which carry heat under the form of water vapor enthalpy, but also carbon dioxide and oxygen absorption and emission which do not directly influence the heat transfers.

The present study restricts itself to heat transfers, and the subsequent thermal conditions observed in the vessels; therefore, it is not essential to distinguish between evaporation from the gel and evaporation by the plants. For the same

reason, O_2 and CO_2 gas exchanges can be safely ignored in the model. Furthermore, it is possible to entirely base the description of water mass transfers on the assumption of similitude between heat and mass transfers (the so-called Lewis hypothesis), so that a precise description of the convective mode is sufficient to determine all parameters of the model. Then, evaporation will occur whenever water vapor partial pressure in the vessel lies below the saturation value, and condensation on walls will take place whenever the wall surface temperature T_p drops below the dew point of the air in the vessel. In these conditions, and neglecting the water vapor specific heat in front of its latent heat of phase change, the global heat exchange (convective and mass transfer) on a surface S reads:

$$\Phi \simeq h_{cv}/CpS[H_s(T_p)-H(T)]$$

where Cp is the specific heat of dry air, $H_s(T_p)$ the enthalpy of saturated air at temperature T_p and H the enthalpy of the humid air inside the vessel; the enthalpy itself is defined as the sum of two terms, one for the air and one for the water content w (expressed in kg per kg of dry air):

$$H(T) = Cp_a T+w\,[L_v(T)+Cp_v T]$$

(in which the subscript a stands for the air and v for the vapor) and H_s being the value of H for the value w_s of w at saturation.

The model described so far gives rise to a set of 5 linear equations: there is one equation for each constituent of the vessel (the stopper, the wall, the bottom, the air inside and the substrate). Each equation expresses the balance between heat and mass gains and heat and mass losses. For example, the heat balance for a given part (denoted by the subscript p) of the vessel reads:

$$\alpha_p S_p R = \sum_i h_{cv}/Cp\ S_p[H_s(T_p)-H(T_i)]+\sum_j h_r S_p(T_p-T_j)+\rho_p Cp_p \mathrm{d}T_p/\mathrm{d}t$$

where the index i runs over the various fluids, and the index j over the various surfaces surrounding the considered wall. R is the normal component of the radiation incident on the surface S_p; ρ_p is the volumic mass and Cp_p the specific heat of that part. The last term, depending explicitly on time, reflects the ability of the model to follow slow fluctuations of outside thermal conditions. In such cases, the model expresses the thermal behavior of a vessel as a succession of quasi-stationary states with only linear temperature gradients inside the walls.

In this set of equations, a few parameters (the mean temperatures involved in the linearization procedure of the radiative exchanges) must be modified at each stage of an iterative procedure before the solutions obtained for the 5 unknowns can be accepted as self consistent. This iterative scheme is well fitted for small computers and converges rapidly to a quasi-stationary state of the vessel.

2. EXPERIMENTAL PROCEDURE

Actual temperature and radiation measurements were carried out in standard micro-propagation units: such units, devoted to Gerbera multiplication, were indoors opaque 20 m^3 cabins, insulated and equipped with temperature and humidity

control devices. Inside these cabins, a set of successive horizontal shelves supported densely packed glass vessels directly placed under racks of fluorescent tubes. The glass vessels, 0.105 m high and 0.085 m in diameter, were closed by polycarbonate transparent stoppers which are normally not air tight in practical applications, but were here sealed with silicon. The fluorescent tubes were 58 W Sylvania White gas discharge tubes of 0.023 m diameter and 1.5 m length, with 0.21 m spacing between them, which deliver less than 20 W/m^2 of short wave radiations on the top of the vessels for an electric load of 40 W per tube (a 10% efficiency).

For each instrumented vessel, temperature probes (100 Ohm platinum resistors Pt M3 and EPS 100 C20 from Comptoir Lyon Allemand Louyot), were placed on the lateral walls, the bottom and the cover, and also inside the gel and in the confined air. The probes were protected against direct radiation by aluminized tape; the thermal contact of surface sensors was promoted by means of Silicojelt 13. All probes were connected to a digital Ohm-meter TN2C from A.O.I.P. through a BCM2 P11 NP8 switch box. The short wave length radiation was measured with a corrected Silicon cell Solar 118 from HAENNI. Air velocities were measured with hot wire anemometers TA400 from Air Flow Devel. Ltd. In the first experiment, a representative vessel (situated in the center of one shelve, among identical vessels) received a normal culture gel (water and agar, at 8 g/l, plus sucrose, at 25 g/l) and represented a case with no plantlet.

In the second experiment, the presence of a plantlet was simulated by the addition of active charcoal to the culture gel, resulting in a measured absorptivity of 95% in the solar radiation spectrum.

The third experiment involved a different type of glass vessel, of 0.130 m height and 0.105 m diameter, placed on expansed polystyrene opaque trays. Powerful sodium discharge bulbs, delivering a much larger photosynthetic radiation intensity than fluorescent tubes, were placed behind a glass sheet above the vessels and were actively ventilated to prevent overheat from far infrared emissions. The relevant physical parameters for these three experiments are summarized in Table 1. Reflexivity and absorptivity were measured with a Varian 17 D spectro-photometer (see Fig. 2). The thermal conductivity of the gel was estimated with the method of Bruckler (*1*).

In all experiments, a thin oil film was poured on the gel to cancel all potential water evaporation. This was done to create experimental conditions able to validate the convective coefficients used in the model.

3. EXPERIMENTAL RESULTS AND CALCULATED TEMPERATURES

Air velocity measurements performed between the vessels, with values ranging from 0.2 to 0.4 m/s, indicated that laminar free convection correlation expressions would be best suited for the present experimental situation ($Nu \simeq 0.43 Gr^{1/4}$).

The Table 2 shows the comparison between measured and calculated temperatures. The main features are the following:

Temperature gradients appear to be very weak (a 0.5°C difference at most in

Table 1. Physical parameters for each experiment

Set up No.		1	2	3
Outside air (room) temperature	(°C)	22.3	21.6	28.7
Surface temperature of fluorescent tubes	(°C)	38.9	37.6	—
Surface temperature of glazing	(°C)			35.0
Shortwave radiations coming downwards	(W/m^2)	17	16	97
Shortwave radiations coming upwards	(W/m^2)	7	6.5	0
Vertical wind speed	(m/s)	0.1	0.1	0.3
Horizontal wind speed	(m/s)	0.1	0.1	0.3
Medium optical absorptivity for shortwave radiations	(%)	20	95	11
Medium optical absorptivity for longwave radiations	(%)	100	100	100
Medium optical reflectivity for shortwave radiations	(%)	5	5	5
Medium optical reflectivity for longwave radiations	(%)	0	0	0
Medium thermal conductivity	(W/m°C)	0.6	0.6	0.6
Stopper optical absorptivity for shortwave radiations	(%)	0	0	0
Stopper optical absorptivity for longwave radiations	(%)	100	100	100
Stopper optical reflectivity for shortwave radiations	(%)	5	5	5
Stopper optical reflectivity for longwave radiations	(%)	0	0	0
Glass optical absorptivity for shortwave radiations	(%)	0	0	0
Glass optical absorptivity for longwave radiations	(%)	100	100	100
Glass optical reflectivity for shortwave radiations	(%)	10	10	10
Glass optical reflectivity for longwave radiations	(%)	0	0	0

the first experiment, 7.9°C in the third experiment involving larger radiation intensities). This is in favor of purely laminar free flows along the vessel walls. Such a weak greenhouse effect inside the vessels is the consequence of the very low radiation levels commonly used in the micropropagation units. It clearly leaves room for delivering more intense irradiation levels on the vessels, before any dangerous temperature rise could be created inside. The presence of an opaque material inside the vessel (experiment No. 2) does not result in a noticeable temperature increase of the gel: a real *in-vitro* vessel containing a plantlet would not exhibit a much higher temperature. In addition, as air leaks and evaporation processes are inhibited in these experiments, realistic conditions would give rise to even smaller temperature gradients.

The model gives temperature predictions with a good accuracy: discrepancies of 0.1 to 0.2°C are observed, which represent a relative error of 1%, generally speaking. Given the restricted range of actual temperatures, it is not possible to

Table 2. Comparison between measured and simulated values

First set up

	Measured values (°C)	Simulated values (°C)	Discrepancy	
			abs. (°C)	rel. (%)
Temperature of stopper	23.4	23.4	0	0
Temp. of vessel surface	23.0	22.8	0.2	1.25
Temp. of air inside	22.9	22.9	0	0
Temp. of medium surface	22.9	23.0	0.1	0.63
Temp. of vessel bottom	22.9	23.0	0.1	0.63

Second set up

	Measured values (°C)	Simulated values (°C)	Discrepancy	
			abs. (°C)	rel. (%)
Temperature of stopper	22.9	22.9	0	0
Temp. of vessel surface	22.3	22.3	0	0
Temp. of air inside	22.6	22.6	0	0
Temp. of medium surface	23.2	23.3	0.1	0.65
Temp. of vessel bottom	23.2	23.3	0.1	0.65

Third set up

	Measured values (°C)	Simulated values (°C)	Discrepancy	
			abs. (°C)	rel. (%)
Temperature of stopper	30.2	30.1	0.1	0.8
Temp. of vessel surface	22.3	22.3	0	0
Temp. of air inside	22.6	22.6	0.1	0.8
Temp. of medium surface	23.2	23.3	0.05	0.4
Temp. of vessel bottom	23.2	23.3	0.1	0.8

The relative discrepancy is calculated relatively to the maximum observed temperature gradient in the experiment.

Table 3.

A: Common physical conditions taken in the various simulations on the effect of increased irradiance on the leaf temperature

Outside air temperature	28.5 °C
Temperature of the array surface	32.0 °C
Vertical wind speed	0.1 m/s
Horizontal wind speed	0.2 m/s
Air exchange rate in the vessels	0.6 vol/h

B: Result of simulations for different incident radiation levels

	Radiation (W/m^2)	Leaf temperature (°C)
Condition No. 1	100	34.9
Condition No. 2	200	41.2
Condition No. 3	300	47.6
Condition No. 4	400	53.7
Condition No. 5	500	59.9

really qualify the model in more details. It is however possible to use the numerical model to simulate now different physical environments and to include the effect of evaporation and condensation processes inside the vessels. This is what is presented in Table 3. This simulation was carried out on the same set of vessels as in the experiment 2, but different values are affected to the outside air temperature, the stopper surface temperature, the vertical and the horizontal wind speed, the mass exchange rate of the vessel and the shortwave radiation coming downwards. The relative humidity of the ambiant air is assumed to be 80%. Longwave radiation exchanges are neglected (especially with the sky). They would only contribute to reduce the simulated temperatures.

Situations where the radiation level is much higher than the 10 to 30 W/m^2 level considered so far rise in fact serious practical difficulties from an experimental point of view: the radiative contribution on temperature probes cannot be easily suppressed in small vessels, and this leads to large over-estimates of the air or of surface temperatures. On the contrary, the model maintains its relative accuracy in all conditions and offers a more reliable estimate of actual temperatures in a confined vessel. From this approach, it is possible to deduce a value of acceptable incident radiation inside a glass vessel which should not endanger the vegetal inside. Under the hypothesis of the present calculations, it can be seen that the maximum radiation level that plantlets can support lies between 200 and 300 W/m^2. These values can be achieved in glasshouses, provided a shading screen is used under bright sun.

CONCLUSION

The model developed above, which includes radiative transfers, convective exchanges and water vapor mass transfers, is able to describe the thermal behavior of micropropagation transparent vessels submitted to various thermal conditions. It was applied to assess stationary heat transfers between a vessel and its environment, which are the most usual case of application. But it could also be used to describe slowly varying physical situations like those *in-vitro* culture units would face in natural light conditions. In the present context, it is shown that one could apply much higher radiation levels than usually found in classical micropropagation units, with potential benefits in terms of biological process rates, without creating dangerous thermal stress for the plants. Other parameters, like the carbon dioxide and various gas concentrations inside the vessels, should then be considered, and a precise monitoring of them could be the next step for optimizing the biological productivity of micropropagation units.

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