[Letter to the editor] Ethylene emitted by nylon membrane filters questions their usefulness to transfer plant seedlings between media

Transfer of seedlings between media is a time-consuming process because the traditional, widely used method involves using forceps to transfer the plantlets one by one. The previously reported solution to avoid such a laborious procedure is the use of nylon membrane filters placed onto the surface of a medium. Despite the increasing popularity of this approach, justification for choosing nylon membranes and their evaluation as a tool for such methods have not been performed yet. In this work we have shown that wet nylon filters, when placed on the surface of the medium, emit significant amounts of ethylene, a gaseous phytohormone which plays a major role in many aspects of plant development and their response to environmental signals. Therefore, our results indicate that the use of nylon membranes as a material for seedling growth support may be problematic due to the risk of introducing an additional, uncontrolled factor during experiments.

Transfer of seedlings between media is a time-consuming process, particularly when using forceps to relocate plantlets one by one. This technique can result in the generation of biological responses in the seedlings to touch and wounding. Therefore, it has to be done carefully, which requires time and qualified, manually skilled practitioners. Being aware of these facts, it is not surprising that alternative methods to reduce the time required and eliminate the possibility of the stress-inducing treatment have been searched for. One of the reported solutions is the use of nylon membrane filters placed on the surface of solid growth medium as a support for seedlings (1). Nowadays, more research groups have been using nylon filters, mainly for the harvesting of roots but also for transferring seedlings between media (2–12). Despite the increasing popularity of this approach, nylon membrane filters have never been characterized in terms of their specific effects on plants.

The main attributes of a material for seedling support should be the lack of interference with plant growth and metabolism. Nylon seems to have such properties because no apparent difference could be observed between plants grown on nylon filters (placed on the growth medium) and directly on the medium (Figure 1). The absence of observable growth disturbances is a necessary requirement but it is not enough in the case of more sophisticated research techniques detecting subtler changes in the transcriptome, metabolome and proteome of plants. In many experiments, even substantial changes in plant metabolism were not manifested visually early on. Therefore, there is a strong need to determine if nylon filters could contaminate media through the emission of any biologically active factors. These special considerations were prompted by the recent report of biologically active contaminants leaching from some disposable plasticware, which disqualifies them from some applications (13).

It is noteworthy that water elutes cyclic polyamide oligomers from nylon (14). Such extractables are not biologically active; however, the aim of that research was to identify contaminants affecting chromatography, hence only high-molecular weight compounds were investigated. On the other hand, low-molecular weight compounds may have greater biological activity. One example of such a small compound that is a common contaminant released from many synthetic polymers is ethylene (also known as ethene). Ethylene is a gaseous plant hormone that regulates many processes of plant growth and development, such as seed germination, seedling growth, fruit ripening, organ development, senescence, and abscission. It also plays an important role in plant responses to stresses such as drought, waterlogging, flooding, wounding, salinity, and various pathogens (15,16). Ethylene therefore has a wide spectrum of the biological functions and is able to change plant metabolism when present even at trace levels. The observation that ethylene occurs as an impurity in synthetic polymers suggested that nylon could also emit this compound. To clarify this problem we decided to test if nylon filters can produce measurable amounts of ethylene. For this purpose a commercial laser-based photoacoustic ethylene detector (type ETD-300, Sensor Sense B.V., The Netherlands) was used due to its simplicity. Such an ethylene detector is able to measure in real time about 300 pptv (parts-per-trillion volume) of ethylene within 5 s. This detector can be combined with an in-line sampling system (type VC-6, Sensor Sense B.V., The Netherlands),to measure up to six cuvettes per experiment (17), which allows for the measurement

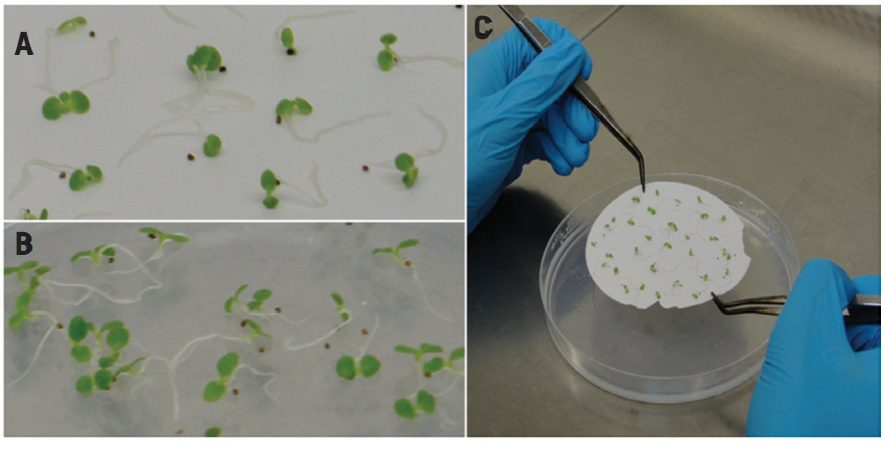

Figure 1. Transfer of plant seedlings using nylon membranes. Nylon filters appear to have no obvious, visible effects on plant growth; compare the seedlings grown on a nylon filter placed on medium (A) and the seedlings grown directly on medium without a filter (B). The filters allow easy, fast, and non-contact seedling transfer between media (C).

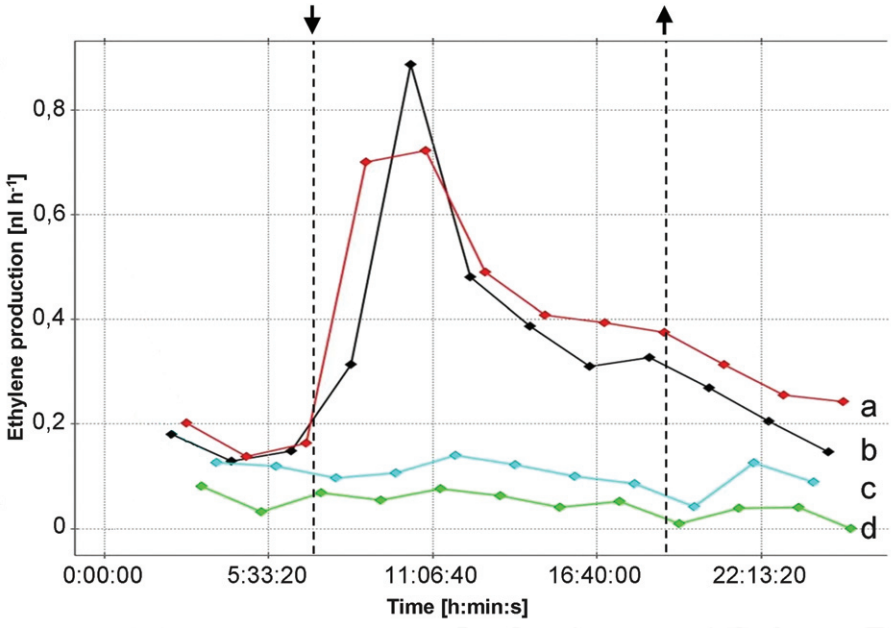

Figure 2. Ethylene production by nylon filters. Data from experimental cuvettes with nylon filters placed on plates with plant growth medium (a, b) are shown and compared with the controls without nylon membranes (c, d). The arrows indicate the time points for placing (↓) and removing (↑) nylon filters in cuvettes a and b.

of control and experimental samples at the same time under identical conditions. The experiment was performed using the automated VC-6 sampling system set up to operate in "stop and flow" mode. Before placing the Petri dishes containing AB medium (18) into the sampling cuvettes they were filled with glass beads to minimize the headspace volume. Next, the cuvettes were hermetically sealed and fitted with inlet and outlet ports. Ethylene was allowed to accumulate in the headspace for 90 min before transporting to the ETD-300 detector where it was measured alternately for 30 min for each cuvette. All parts of the equipment used in this approach — glass beads, glass cuvettes, and tubing — were checked for ethylene production, with negative results. For the measurement of ethylene production, nylon membrane (Whatman, Nytran N, 0,45 μm) circles of 9 cm in diameter were placed onto the surface of the AB medium in two out of four Petri dishes used in the experiment. All four cuvettes were controlled simultaneously; while one was being measured, the others were accumulating gases to optimize the efficiency of measurement. Ethylene production was calculated as the ethylene emission rate [nl h^{-1}] by multiplying the measured values (in ppbv) by the flow rate.

The data clearly indicated that nylon filters, placed on the surface of the media, emitted significant amounts of ethylene (Figure 2). Placing the filters on the media resulted in at least a four-fold increase of ethylene in comparison to the controls, which were Petri plates with AB media, but without filters. A comparable level of ethylene is produced by plants exposed to environmental challenges such as nutrient limitation (19) and could affect gene expression. In addition, exogenous ethylene at concentrations as low as a few nanoliters per liter were reported to modulate root waving, root growth direction, and looping in Arabidopsis (20). After removing the filters from the cuvettes, the ethylene level decreased during successive flow steps; however, it did not reach the level measured from plates without filters. We subsequently observed that spraying the nylon membrane with demineralized water was sufficient to stimulate ethylene emission (data not shown), which excludes the involvement of any medium ingredients in this ethylene burst. Interestingly, dry nylon filters placed into dry empty dishes did not emit measurable amounts of ethylene (data not shown). We need to emphasize that in this experiment no seedlings were grown and no plant response to the ethylene was investigated. However, one can expect that, due to a free diffusion of this gaseous compound, seedlings grown on a nylon filter placed on medium in a Petri dish could be exposed to different ethylene concentrations depending on the material (porous or not) used for sealing of the dish.

In summary, our results suggests that great caution should be exercised in using nylon as a material for forming the surface and support for seedling growth, due to the possibility of introducing an additional, uncontrolled factor during experiments, namely exposing seedlings to a significant level of ethylene. Moreover, this observation suggests that some previously published results should be reconsidered because they could have been affected by ethylene emitted by nylon filters. Identification of filters that do not emit ethylene would be useful for methods requiring mechanical support for seed germination and seedling growth.

Acknowledgments

This work was supported by the Ministry of Science and Higher Education from Poland (1194/B/P01/2008/35) and by EU-FP6-Infrastructures-5 Program (FP6-026183).

Competing interests

The authors declare no competing interests.

References

1. **Poirier, Y., S. Thoma, C. Somerville, and J. Schiefelbein.** 1991. Mutant of Arabidopsis deficient in xylem loading of phosphate. Plant Physiol. 97:1087-1093.
2. **Arroyo, A., F. Bossi, R.R. Finkelstein, and P. Leon.** 2003. Three genes that affect sugar sensing (abscisic acid insensitive 4, abscisic acid insensitive 5, and constitutive triple response 1) are differentially regulated by glucose in Arabidopsis. Plant Physiol. 133:231-242.
3. **Farras, R., A. Ferrando, J. Jasik, T. Kleinow, L. Okresz, A. Tiburcio, K. Salchert, C. del Pozo, et al.** 2001. SKP1-SnRK protein kinase interactions mediate proteasomal binding of a plant SCF ubiquitin ligase. EMBO J. 20:2742-2756.
4. **Zentella, R., Z.L. Zhang, M. Park, S.G. Thomas, A. Endo, K. Murase, C.M. Fleet, Y. Jikumaru, et al.** 2007. Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. Plant Cell 19:3037-3057.
5. **Li, L., S.M. Li, J.H. Sun, L.L. Zhou, X.G. Bao, H.G. Zhang, and F.S. Zhang.** 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc. Natl. Acad. Sci. USA 104:11192-11196.
6. **Tomscha, J.L., M.C. Trull, J. Deikman, J.P. Lynch, and M.J. Guiltinan.** 2004. Phosphatase under-producer mutants have altered phosphorus relations. Plant Physiol. 135:334-345.
7. **Cernac, A., C. Andre, S. Hoffmann-Benning, and C. Benning.** 2006. WRI1 is required for seed germination and seedling establishment. Plant Physiol. 141:745-757.
8. **Takano, J., M. Wada, U. Ludewig, G. Schaaf, N. von Wiren, and T. Fujiwara.** 2006. The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. Plant Cell 18:1498-1509.
9. **Engineer, C.B., K.C. Fitzsimmons, J.J. Schmuke, S.B. Dotson, and R.G. Kranz.** 2005. Development and evaluation of a Gal4-mediated LUC/GFP/GUS enhancer trap system in Arabidopsis. BMC Plant Biol. 5:9.
10. **Kataoka, T., N. Hayashi, T. Yamaya, and H. Takahashi.** 2004. Root-to-shoot transport of sulfate in Arabidopsis. Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. Plant Physiol. 136:4198-4204.
11. **Knowles, S.M., S.X. Lu, and E.M. Tobin.** 2008. Testing time: can ethanol-induced pulses of proposed oscillator components phase shift rhythms in Arabidopsis? J. Biol. Rhythms 23:463-471.
12. **Nikiforova, V., J. Freitag, S. Kempa, M. Adamik, H. Hesse, and R. Hoefgen.** 2003. Transcriptome analysis of sulfur depletion in Arabidopsis thaliana: interlacing of biosynthetic pathways provides response specificity. Plant J. 33:633-650.
13. **McDonald, G.R., A.L. Hudson, S.M. Dunn, H. You, G.B. Baker, R.M. Whittal, J.W. Martin, A. Jha, et al.** 2008. Bioactive contaminants leach from disposable laboratory plasticware. Science 322:917.
14. **Tran, J.C. and A.A. Doucette.** 2006. Cyclic polyamide oligomers extracted from nylon 66 membrane filter disks as a source of contamination in liquid chromatography/mass spectrometry. J. Am. Soc. Mass Spectrom. 17:652-656.
15. **Adie, B., J. Chico, I. Rubio-Somoza, and R. Solano.** 2007. Modulation of plant defenses by ethylene. J. Plant Growth Regul. 26:160-177.
16. **Lin, Z., S. Zhong, and D. Grierson.** 2009. Recent advances in ethylene research. J. Exp. Bot. 60:3311-3336.
17. **Cristescu, S.M., S.T. Persijn, S. te Lintel Hekkert, and F.J.M. Harren.** 2008. Laser-based systems for trace gas detection in life sciences. Appl. Phys. B 92:343-349.
18. **Lewandowska, M., A. Wawrzynska, G. Moniuszko, J. Lukomska, K. Zientara, M. Piecho, P. Hodurek, I. Zhukov, et al.** 2010. A contribution to identification of novel regulators of plant response to sulfur deficiency: characteristics of a tobacco gene UP9C, its protein product and the effects of UP9C silencing. Mol Plant 3:347-360.
19. **Zuchi, S., S. Cesco, Z. Varanini, R. Pinton, and S. Astolfi.** 2009. Sulphur deprivation limits Fe-deficiency responses in tomato plants. Planta 230:85-94.
20. **Buer, C.S., G.O. Wasteneys, and J. Masle.** 2003. Ethylene Modulates Root-Wave Responses in Arabidospsis. Plant Physiol. 132:1085-1096.

Received 20 July 2011; accepted: 21 September 2011.

Grzegorz Moniuszko[1], Aleksandra Laska-Oberndorff[2], Simona M. Cristescu[2], Frans J.M. Harren[2], Agnieszka Sirko[1]

[1]Department of Plant Biochemistry, Institute of Biochemistry and Biophysics PAS, Pawinskiego 5A, 02-106 Warsaw, Poland
[2]Life Science Trace Gas Facility, Molecular and Laser Physics, Institute of Molecules and Materials, Radboud University, Heyendaalseweg 135, The Netherlands

Address correspondence to Agnieszka Sirko, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawinskiego 5A, 02-106 Warsaw, Poland.
e-mail: asirko@ibb.waw.pl

BioTechniques 51: 329-332 (November 2011)
doi 10.2144/000113762