

Effect of ethylene and its antagonist 1-MCP on the senescence of detached leaves of *Arabidopsis thaliana*

V.S. ALEXIEVA*¹, I.G. SERGIEV*, D.A. TODOROVA*, E.N. KARANOV*, A.R. SMITH** and M.A. HALL**

Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences,

*Acad. G. Bonchev Str., Bl. 21, BG-1113 Sofia, Bulgaria**

Institute of Biological Sciences, University of Wales,

*Edward Liwyd Bl., SY233DA Ceredigion, Aberystwyth, UK***

Abstract

1-Methylcyclopropene (1-MCP) applied alone did not influence significantly the chlorophyll and carotenoid content of the older leaves of *Arabidopsis thaliana* (L.) Heynh., but retarded the senescence of the younger ones (6th and 7th leaf nodes). However, 1-MCP effectively blocks the ethylene induced senescence of excised rosette leaves. The preliminary application of 1-MCP (3 h in advance to the treatment by *Ethrel*) almost totally eliminated the ethylene action. Similar trend was also observed after simultaneous application of *Ethrel* and 1-MCP, and the effects of both treatments on the chlorophyll and carotenoid destruction are comparable.

Additional key words: carotenoids, chlorophyll, ethylene releasing agent, leaf node.

The leaf yellowing is one of the first visible symptoms of senescence – natural or induced by various unfavourable environmental factors (Thomas and Stoddart 1980, Zacarias and Reid 1990). It is well known that the ethylene production is enhanced during the leaf ageing (Nichols 1966) and this process can be accelerated by the application of ethylene (Matoo and Aharoni 1988, Zacarias and Reid 1990). On the other hand there are number of compounds which can diminish the ethylene effects – cytokinins, Ag-thiosulphate, CO₂, nitric oxide, nitrous oxide, 2,5-norbornadiene, cyclooctene, cyclooctadiene, some derivatives (esters and hydrazides) of the dicarboxylic acids, etc. (Sisler and Pian 1972, Alexieva 1987, Goldthwaite 1988, Sisler *et al.* 1990, 1996, Leshem and Wills 1998). Recently, 1-methylcyclo-propene (1-MCP) is a wide used as one of the strongest ethylene antagonist (Sisler *et al.* 1996, Zhong *et al.* 2001).

The aim of this study was to establish the effectiveness of 1-MCP in preventing the senescence of detached leaves of wild type *Arabidopsis thaliana* caused

by the ethylene releasing agent *Ethrel* (2-chloroethyl-phosphonic acid).

A wild type of *Arabidopsis thaliana* (L.) Heynh, cv. Columbia were used. The plants were grown in plastic pots (d = 70 mm, h = 80 mm), filled with soil/*Perlite* mixture (3:1) in a growth chamber (16-d photoperiod, 70 μmol m⁻² s⁻¹ photon flux density, 24/20 °C day/night temperature, 60 % air humidity). The plants were daily irrigated. Rosette leaves (3rd to 7th leaf node) from 30-d-old plants were cut, weighed and placed in Petri dishes (d = 100 mm) for incubation on two layers of filter paper wetted with 5 cm³ of distilled water (control) or *Ethrel* (0.001 cm³ dm⁻³). The 1-MCP treatment was performed for 3 h (prior to, or along with the application of *Ethrel*) by injection (0.00012 cm³ dm⁻³) in a sealed glass chambers containing a set of excised leaves in Petri dishes. After that all samples were incubated in darkness (25 °C) for 48 h and chlorophyll and carotenoid content was measured spectrophotometrically (Arnon 1949). 1-MCP was synthesized

Received 17 September 2003, accepted 15 April 2004.

Abbreviations: Car - carotenoids; Chl - chlorophyll; 1-MCP - 1-methylcyclopropene

Acknowledgements: This work was supported by the INCO COPERNICUS Programme ERBIC15CT960914.

¹ Corresponding author; fax: (+359) 2739952, e-mail: vereva@obzor.bio21.bas.bg

according to Magid *et al.* (1971). The results obtained are from two experiments, in three replications each. The data presented are means \pm standard errors (SE).

In our model system, the ethylene releasing agent in the applied concentration enhanced the chlorophyll (Fig. 1A,B) and carotenoid (Fig. 1C) destruction of all leaf nodes. It was established that the chlorophyll destruction in naturally (Sergiev *et al.* 2003, Todorov *et al.* 2003a) and induced by extreme temperatures (Todorov *et al.* 2003b) senescing leaves of *Arabidopsis thaliana* was more pronounced in the wild type plants than in the ethylene insensitive mutant *eti5* plants.

Applied alone the ethylene antagonist did not influence significantly the chlorophyll and carotenoid content of the older leaves, but retarded the senescence of the younger ones (6th and 7th leaf nodes). The preliminary application of 1-MCP (3 h in advance to the treatment by *Ethrel*) almost totally eliminated the ethylene action. Similar trend was also observed after simultaneous application of *Ethrel* and 1-MCP, and the effects of both treatments on the chlorophyll and carotenoid destruction are comparable. Although the mode of action of 1-MCP is still not completely revealed, it is assumed that it binds to the ethylene receptors in an irreversible manner (Sisler *et al.* 1996, Zhong *et al.* 2001). So, it could be supposed

that in our model system 1-MCP acts in a similar manner, and even in a simultaneous application of 1-MCP with the ethylene releasing agent, 1-MCP occupies at least part of the ethylene receptors and that is why there is no a lag-period observed for expression of its antagonistic action. Similar results are reported in other plant species, carnation and citrus (Sisler *et al.* 1996, Zhong *et al.* 2001). According to Sisler *et al.* (1996), the ethylene blocker applied 5 min in advance to ethylene but in a higher concentration ($0.0002 \text{ cm}^3 \text{ dm}^{-3}$), almost completely eliminates the effects of the exogenously applied ethylene. It should be noted that 1-MCP considerably differs in its nature from the other well known agents which express senescence retarding effects (cytokinins, silver thiosulphate, aminooxyacetic acid, *etc.*), as well as from compounds acting as ethylene synergists (ABA for instance). The observed effects of the ethylene blocker 1-MCP on detached *Arabidopsis* rosette leaves, and the fact that its action is better expressed in the younger leaves gives an additional information on the senescence processes of excised leaves. Future investigations of the effects of 1-MCP in ethylene related and other *Arabidopsis* mutants, will contribute for a better understanding of the ethylene mode of action.

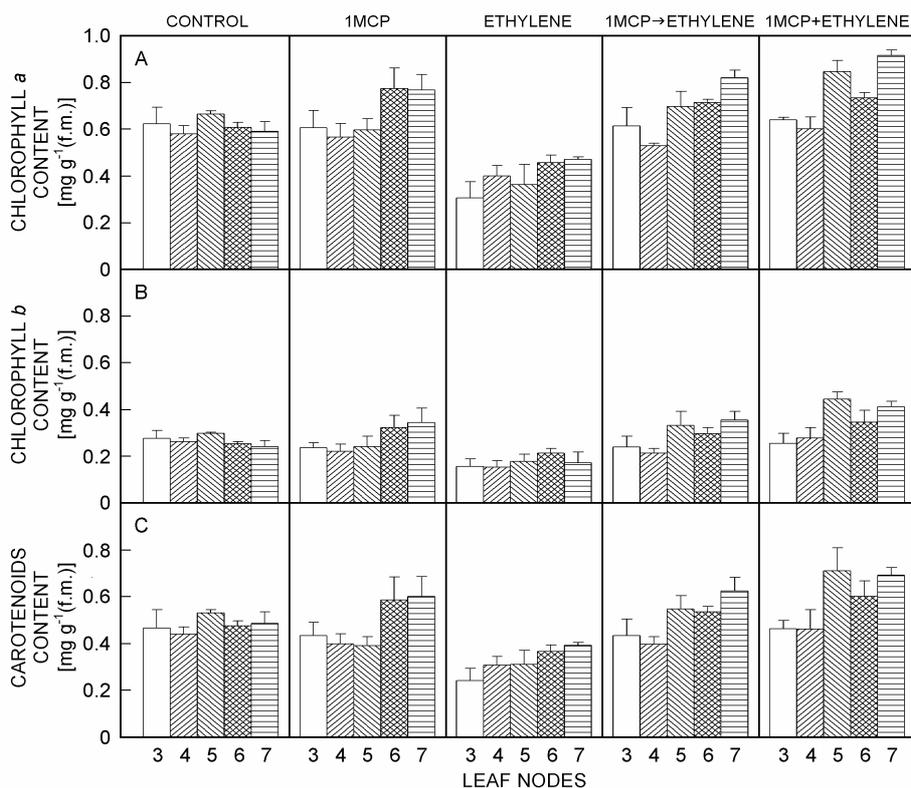


Fig. 1. Effects of ethylene, 1-MCP, and combinations of them on the contents of chlorophyll *a* (A), chlorophyll *b* (B), and carotenoids (C) in excised rosette leaves (leaf nodes 3 to 7) of *Arabidopsis thaliana*, after 48 h of incubation in darkness. Contents of Chl *a*: 3rd - 0.935, 4th - 0.868, 5th - 0.975, 6th - 1.278, 7th - 0.944; Chl *b*: 3rd - 0.449, 4th - 0.623, 5th - 0.456, 6th - 0.634, 7th - 0.430; and Car: 3rd - 0.727, 4th - 0.848, 5th - 0.787, 6th - 1.092, 7th - 0.820 mg g⁻¹(f.m.) before experiment.

References

- Alexieva, V.: [Plant growth regulating activity of some aliphatic dicarboxylic acids and their mono- and di-substituted derivatives.] - Doctoral Thesis, Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia 1987. [In Bulg.]
- Arnon, D.: Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. - *Plant Physiol.* **24**: 1-15, 1949.
- Goldthwaite, J.J.: Hormones in plant senescence. - In: Davis, P. (ed.): *Plant Hormones and their Role in Plant Growth and Development*. Pp. 553-573. Kluwer Academic Publishers, Dordrecht 1988.
- Leshem, Y., Wills, R.: Harnessing senescence delaying gases nitric oxide and nitrous oxide: A novel approach to postharvest control of fresh horticultural produce. - *Biol. Plant.* **41**: 1-10, 1998.
- Magid, R., Clarke, T., Duncan, C.: An efficient and convenient synthesis of 1-methylcyclopropene. - *J. org. Chem.* **36**: 1320-1321, 1971.
- Matoo, A., Aharoni, N.: Ethylene and plant senescence. - In: Nooden, L., Leopold A. (ed.): *Senescence and Aging in Plants*. Pp. 241-281. Academic Press, San Diego 1988.
- Nichols, R.: Ethylene production during senescence of flowers. - *J. hort. Sci.* **41**: 279-290, 1966.
- Sergiev, I., Todorova, D., Alexieva, V., Karanov, E., Smith, A., Hall, M.: Rosette leaf senescence in wild type and ethylene-insensitive mutant of *Arabidopsis thaliana* (L.) Heynh inflorescence and fruit development. - In: Machackova, I., Romanov, G. (ed.): *Proceeding from NATO-Russia Workshop "Phytohormones in Plant Biotechnology and Agriculture"*. Pp. 217-228. Kluwer Academic Publishers, Dordrecht 2003.
- Sisler, E., Blankenship, S., Guest, M.: Competition of cyclooctenes and cyclooctadienes for ethylene binding and activity in plants. - *Plant Growth Regul.* **9**: 157-164, 1990.
- Sisler, E., Dupille, E., Serek, M.: Effect of 1-methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnation. - In: Smith, A., Berry, A., Harpham, N., Moshkov, I., Novikova, G., Kulaeva, O., Hall, M. (ed.): *Plant Hormone Signal Perception and Transduction*. Pp. 127-134. Kluwer Academic Publishers, Dordrecht 1996.
- Sisler, E., Pian, A.: Effect of ethylene and cyclic olefins on tobacco leaves. - *Tobacco Sci.* **17**: 68-72, 1972.
- Thomas, H., Stoddart, J.: Leaf senescence. - *Annu. Rev. Plant Physiol.* **31**: 83-111, 1980.
- Todorov, D., Karanov E., Smith, A., Hall, M.: Chlorophyllase activity and chlorophyll content in wild and mutant *Arabidopsis thaliana*. - *Biol. Plant.* **46**: 125-127, 2003a.
- Todorov D., Karanov, E., Smith, A., Hall, M.: Chlorophyllase activity and chlorophyll content in wild and *eti 5* of *Arabidopsis thaliana* subjected to low and high temperatures. - *Biol. Plant.* **46**: 633-636, 2003b.
- Zacarias, L., Reid, M.: Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. - *Physiol. Plant.* **80**: 549-554, 1990.
- Zhong, G., Huberman, M., Feng, X., Sisler, E., Holland, D., Goren R.: Effect of 1-methylcyclopropene on ethylene-induced abscission in citrus. - *Physiol. Plant.* **113**: 134-141, 2001.