Ultrastructural Localization of Silver Deposits in the Receptacle Cells
of Carnation Flowers

H. Veen 1*, S. Henstra 2, and W.C. de Bruyn 3
1 Centre for Agrobiological Research, P.O. Box 14, 6700 AA Wageningen,
2 Technical and Physical Engineering Research Service, Wageningen, and
3 Analytical Electron Microscope Unit**, Laboratory for Electron Microscopy, University of Leyden, Leyden, The Netherlands

Abstract. Carnations were treated with a silver thiosulphate complex to prevent wilting of the flowers. The ultrastructural localization of silver and sulphur in the receptacle tissue was investigated by electron microscopy. Electron-dense deposits were present in the receptacle tissue. Coarse-grained deposits (diam. 60–100 nm) were predominantly observed at the inner side of the cell wall, whereas fine-grained deposits (diam. 20–60 nm) were predominantly present inside the cell-wall region and in the intercellular spaces. These particles were analyzed for chemical elements by X-ray analytical electron microscopy (Philips EM 400 plus Edax energy dispersive analyzer, type 711). In both types of deposits, the presence of silver and sulphur was verified. Point analysis revealed that in both precipitates the S/Ag ratio was of the same order.

Key words: Analytical electron microscopy – Dianthus – Flower longevity – Silver thiosulphate – X-ray.

Introduction

A pretreatment with silver thiosulphate increases the vase-life of cut carnations (Veen and Van de Geijn, 1978). Analysis of the silver content of different flower parts after treatment with silver thiosulphate showed a distinct accumulation in the receptacle tissue. The receptacle tissue is a potential source of high-ethylene production (Veen, 1979). This endogenous ethylene production is fully blocked by a pretreatment with silver thiosulphate. In addition to this effect, such a pretreatment protects the flowers from wilting caused by exogenous ethylene.

As the silver ion acts as a potent antidote to ethylene, it could be used as a tool to find the site of ethylene biosynthesis and its site of action inside the plant cell. Thus, it was decided to study the ultrastructural localization of silver in cells of the receptacle tissue by X-ray analytical electron microscopy.

Materials and Methods

Carnation flowers (Dianthus caryophyllus L. cv. White Sire) were harvested in a commercial nursery, trimmed to a uniform length of 45 cm, and kept out of water overnight at 4°C. Subsequently, the basal ends of the stems were placed into a solution of silver thiosulphate (Ag(S2O3)2-) at a concentration of 2.0 mM, prepared by mixing equal volumes of silver nitrate (AgNO3) and sodium thiosulphate (Na2S2O3·5 H2O) in a concentration ratio of 1:8, respectively. After a 24-h treatment in this solution, the flowers were transferred to deionized water. The experiments were carried out in a greenhouse compartment under natural daylight conditions. The temperature ranged between 16 and 24°C, and the relative humidity between 60 and 70%.

After 6 d, receptacle tissue was isolated from the treated flowers and cut into small blocks of about 1-1.2 mm and fixed overnight at 4°C in 9% glutaraldehyde in Sörensen buffer at pH 7.4 to which some droplets of 0.1 M CaCl2 were added. For general ultrastructural observation, the specimens were washed with the Sörensen buffer, dehydrated with ethanol and embedded in an Epon-Araldite mixture via propylene oxide. Sections 50 nm thick were cut with glass knives on an LKB Ulrotome IV ultramicrotome and stained with uranyl acetate and lead citrate. For observation in the CTEM mode, a Philips EM 300 electron microscope was employed.

For X-ray micro-analysis, the same fixation, dehydration, and embedding procedures were followed. Sections of about 150 nm remained unstained with heavy metal to prevent introduction of foreign elements. Sections were picked up on Formvar-filmed 75 mesh Cu grids and coated with carbon to prevent charging during observation with the analytical electron microscope (Philips EM 400 plus Edax energy-dispersive analyzer, type 711).
For X-ray analysis in the STEM mode, appropriate electron-dense, deposit-containing areas were irradiated for 100 s at an accelerating voltage of 60 kV. With the aid of the stigmator of the condensor lens, the original beam, 100 nm in diameter, was made astigmatic to create an oval spot to cover just as much of the linearly oriented deposits at a magnification of 13,500. Although stray apertures were fitted into the electron microscope to prevent irradiation of the sections by stray X-rays, a spectrum of lifetime 100 s from a hole in the carbon film outside the sections was routinely subtracted from each spectrum by the computer.

The corrected spectra were processed by the ‘ultrathin’ EDAX program of the connected Texas Instruments 16K, Nova 2 computer to reveal the ratios of elements.

Line scans of the sections were imaged with the connected