at densities ranging from 1-2 x 10^6 per ml. The protoplast suspension was adjusted by dilution to a density of 2 x 10^5 per ml. Aliquots of 0.1 - 0.2 ml (20.000 or 40.000 protoplasts) were placed in each well of polystyrene plates containing the toxin solution and incubated without agitation for 72h at 25 °C, in the dark. Seiridins and cyclopaldic acid were assayed at concentrations of 10^{-4}, 10^{-5} and 10^{-6} M. After each incubation time (4 times a day) fluorescein diacetate (FDA) was added to the samples of protoplasts. Number and viability of protoplasts were estimated under a microscope with epifluorescence illumination. FDA staining for viability, which is based on the integrity of the cell membrane, proved a sensitive assay for the toxic compounds used in this study. For demonstration of mitochondria activation, protoplasts were placed in a staining solution containing Janus green B.

The results indicated that prolonged exposure of protoplasts to seiridin (10^{-5} or 10^{-6} M) increased the number of protoplasts and induced changes in their shape. *Iso*-seiridin (10^{-4} M) was less effective. The exposure of protoplasts to cyclopaldic acid (10^{-5} M or less) reduced the uptake of FDA into the cells. Moreover, cyclopaldic acid interfered with mitochondrial activity. These results can be explained through the high chemical reactivity of the ortho difformyl groups adjacent to the carboxyl function of cyclopaldic acid molecule.

CYCLOPALDIC ACID PRODUCTION BY TWO STRAINS OF *SEIRIDIUM CUPRESSI*

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Seiridium cupressi (teleomorph: *Lepteutypa cupressi*) is a pathogenic fungus which causes a destructive canker disease of cypress in various parts of the world. Recent examination of morphological, cultural and physiological characteristics of two strains of *S. cupressi* isolated from cankered cypresses in Greece and in Australia led to the conclusions that the two strains appear to be distinct subspecific entities. The present study shows that both the Greek and the Australian strains produce the major toxic metabolite cyclopaldic acid in culture.

Both fungal strains were grown at 23 °C for one month in the dark on Czapek's medium containing 2% corn meal and, in the case of Australian strain, amino acids and vitamins. The culture filtrates were acidified and extracted with *tert*-butylmethylether. After evaporation of the solvent, the solid mixed to an oily residue was washed with chloroform and then chromatographed in two steps on silica gel plates, using two organic solvents. The spots formed on the chromatogram were visualized with a Fast red salt B solution. The toxin yield was 32-50 mg/l for the Greek strain, and 12-25 mg/l for the Australian strain.