implies the development of selective systems easily and clearly differentiating treated individuals from controls.

Selection procedures based on the action of FC on germination (stimulation of germination, or counterinhibition of ABA effect) proved to be unsuitable because the toxin at all concentration tested (from 10^{-7} M to 10^{-3} M) does not allow a clear discrimination between treated and control seeds. The same is true when the loss of turgor elicited by FC on plants exposed to water stress is evaluated.

More promising seem procedures based: a) on the indirect evaluation of the increased transpiration rate induced by FC, and b) on the synergistic effect of the toxin with the herbicide Paraquat.

Stomatal opening induced by FC (10^{-5} M) on well watered plants incubated at 25 °C in the dark for 12h entertaines a transpiration rate three times higher than that of control plants. Since evapo-transpiration is the major component in the energy balance of leaf, stomatal opening results in cooling of the leaf surface (about 1 °C of difference between treated and control plants) which can be easily visualized with infrared thermography.

The second approach takes the advantage of the synergistic effect of FC with Paraquat. The herbicide, a lipophilic cation, by competing with ferredoxin for electrons from PS 1 and subsequent reoxidation by molecular oxygen, generates superoxide radicals which induce leaf bleaching. We found that in *Arabidopsis*, Paraquat at low doses (5×10^{-7} M) does not induce leaf bleaching after light exposure unless FC is present in the incubation medium. This might depend on a higher Paraquat uptake by the tissue treated with FC or on some metabolic change induced by the toxin. By this selection procedure, 11 seedlings among about 1.200 M2 plants have been selected for resistance to leaf bleaching induced by FC and Paraquat. M3 progenies of these seedling are actually under analysis for the confirmation of the phenotype and for the determination of the physiological basis of resistance.

EFFECT OF SEIRIDINS AND CYCLOPALDIC ACID ON TOBACCO PROTOPLASTS

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Several phytotoxins are produced *in vitro* by the species of *Seiridium* associated with canker diseases of cypress trees in the Mediterranean area. Seiridin and *iso*-seiridin are produced by *Seiridium cardinale*, a strain of *S. cupressi* and *S. unicorne*. In addition, the second species produces a more potent toxin, cyclopaldic acid. The present work was aimed to assay the effect of the three fungal metabolites on plant protoplasts.

Protoplast preparations were made from sterile shoot cultures of *Nicotiana plumbaginifolia*. The protoplasts were resuspended in a buffered mannitol solution

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.