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GIORNATA DI STUDIO DEDICATA ALLA CHIMICA ORGANICA DELLE MOLECOLE BIOLOGICAMENTE ATTIVE

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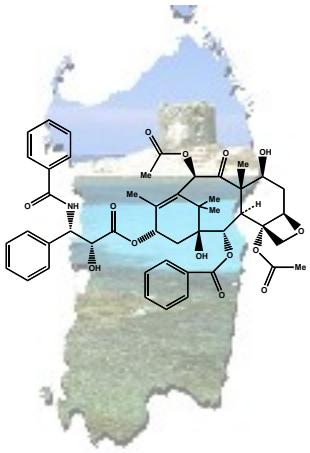

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PHYTOTOXIC METABOLITES PRODUCED BY FUNGI INVOLVEDIN CORK OAK DECLINE

L. Maddau[1], E. Spanu[1], B. T. Linaldeddu[1], A. Franceschini[1] and A. Evidente[2]

[1]*Dipartimento di Protezione delle Piante, Università di Sassari, 07100 Sassari*

[2]*Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli Federico II, 80055 Portici*

Corresponding author: L. Maddau; Phone: +39.079.229368;

Diplodia corticola, anamorph of *Botryosphaeria corticola* Phillips, Alves *et* Luque, and *Biscognauxia mediterranea* (De Not.) O. Kuntze (= *Hypoxylon mediterraneum*) have often been associated with serious decline phenomena, which have been affecting the cork oak forest in Italy and other Mediterranean countries for several years. *Diplodia corticola* is widespread in Sardinian oak forests, and can affect plants of different ages, inducing symptoms which include dieback, cankers and vascular necrosis.

B. mediterranea is also very important in the ethiology of cork oak decline. In particular, when the host plants are exposed to hydric stress, the fungus causes the drying up of the woody organs and the appearance of lesions commonly called "charcoal cankers". The nature and appearance of symptoms caused by these pathogens suggest that phytotoxic metabolites are produced in the cankered tissues, as was also observed for isolates of *D. mutila* from cypress and other oak.

The main phytotoxin isolated from *D. corticola* has been identified as a new monosubstituted tetrahydropyranpyran-2-one, named diplopyrone. Diplopyrone, assayed at concentrations ranging from 0.01 to 0.1 mg/mL was toxic to *Q. suber*. When it was assayed on tomato cuttings, phytotoxicity was evident at 0.2 and 0.1 mg/mL, inducing internal tissue collapse on the stem. The non empirical assignment of its absolute configuration was approached by two different methods. In addition to diplopyrone other metabolites with phytotoxicity activity have been isolated: sphaeropsidins A-C and sapinofuranone B.

Moreover, the same fungus produces: the (*S*,*S*)-diastereomer of sapinofuranone B, (*3S,4R*)-*trans*- and (*3R*,*4R*)-*cis*-4-hydroxymellein, diplobifuranylones A and B, and two new 4-monosubstituted 2(3*H*)-dihydrofuranones, named diplofuranones A and B.

From culture filtrates of *B. mediterranea* three metabolites were isolated uo to now: 5-methylmellein, phenylacetic acid (isolated for the first time as toxic metabolite from this fungus and, finally, a new phytototoxic hexasubstituted pyranopyran, named biscopyran. All the substances were shown to be non-selective toxins.

When absorbed by tomato and oak cuttings, 5-methylmellein at a concentration of 0.1 mg/mL produced wilting and necrosis, respectively. At the same concentration, the growth of the tomato rootlet was inhibited.

Phenyl acetic acid, tested at concentrations ranging from 0.001 to 0.1 mg/mL, was toxic *to Q. suber*. Necrotic lesions appeared on the leaves within 5 days after absorption of the toxic solutions. The cork oak cutting wilted within 10 days at 0.1 mg/mL. When phenylacetic acid was assayed on tomato cuttings, phytotoxicity was observed at 0.1-0.01 mg/mL, inducing internal tissue collapse on the stem.

Biscopyran was assayed on cork oak cuttings causing epinasty at 0.01 and 0.1 mg/mL. The herbaceous plant was also affected by the toxin: wilting appeared on the tomato cuttings at 0.1 and 0.05 mg/mL.

These studies may provide information which could be useful for understanding the chemistry and the biology governing the relationship between these fungi and their hosts. Further studies should aim to also evaluate the ecological role of these substances.