

been evaluated as time course (during the first 60") of water efflux from seeds previously loaded for 1h with $^3\text{H}_2\text{O}$, and by plotting the expression $\ln [(a_t - a_\infty)/(a_0 - a_\infty)]$ versus time. a_0 , a_t , and a_∞ are the radioactive counts associated with the seeds at time = 0, t , and infinity, respectively (W.D. Stein, *Transport and Diffusion across Cell Membranes*, Academic Press, 1986, p. 59). The results show that the presence of 10^{-5} M FC during the first 42h of incubation diminished the permeability of plasmalemma to water: the gradient of the regression line correlating the values of $\ln [(a_t - a_\infty)/(a_0 - a_\infty)]$ with the time of efflux was $b = -0.0066$ in seeds incubated in water and $b = -0.0045$ in seeds incubated with FC. To evaluate whether this effect was linked to the presence of the toxin or to different conditions taking place during germination, due to the action of the toxin, we administered 10^{-5} M or 10^{-4} M FC during the phase of efflux to seeds incubated 42h in water. No immediate effect of FC was detectable. We also performed some experiments using D_2O (50%, v/v) labeled with $^3\text{H}_2\text{O}$ for both loading and unloading the seeds, hypothesizing a different permeability of the plasmalemma to D_2O , what would have allowed us to better evidenciate eventual effects of FC. Though the plasmalemma showed a lower permeability to D_2O ($b = -0.0054$), there was no effect linked to the presence of either 10^{-5} M or 10^{-4} M FC during the efflux phase. The results seem to indicate that when FC is administered to the seeds for long times (42h), it can modify the water permeability characteristics of the plasmalemma. Whether this effect is directly linked to the presence, in the time, of the molecule, or is the consequence of processes (probably, plasticization of cell walls and decrease in cell turgor) promoted by the toxin, is to be ascertained.

FUSICOCIN RECEPTOR: MONOMERIC OR MULTIMERIC PROTEIN?

P. ADUCCI,¹ A. BALLIO,² V. FOGLIANO,¹ M.R. FULLONE,² M. MARRA¹ and D. VERZILI²

¹ Dipartimento di Biologia, Università di Roma «Tor Vergata».

² Dipartimento di Scienze Biologiche, Università di Roma «La Sapienza».

In the study of the mode of action of fusiocin (FC), the mechanism of activation of H^+ -ATPase by the interaction of FC with specific receptors in the plasma membrane has become particularly interesting. Purification of these binding proteins is therefore desirable for understanding the nature of the signal triggered by FC. Attempts so far reported by other authors have afforded preparations not yet thoroughly purified. In our laboratory purification of FC binding proteins from maize shoots was performed by five steps of FPLC and HPLC using adsorption, ion exchange and gel filtration columns. The activity of all fractions was monitored by a specific FC binding assay. Most of the activity was lost after the first three steps of purification, possibly because of the removal of components stabilizing the receptors; the addition of suitable detergents overcomes this inactivation.

The specific activity of the purified fractions was increased several thousand times. The SDS-PAGE silver stained profiles of the fractions evidence proteins with molecular weights of 90, 60 and 30 KDa. These results are consistent with a mul-

meric structure of the receptors, as postulated by other authors; anyhow, the possibility of an uncomplete purification, cannot be ruled out. In order to clarify this point other approaches, such as the use of monoclonal antibodies or of photoaffinity labels, are under investigation.

This work was supported by the Italian Ministry of Agriculture and Forestry, the Italian Research Council (CNR) and the Italian Ministry of Education.

PRODUCTION OF ENNIATIN B BY *FUSARIUM AVENACEUM* AND ITS TOXICITY TO *ARTEMIA SALINA* L.

C. ALTOMARE,¹ A. BOTTALICO,² R. CAPASSO,³ A. EVIDENTE,³ A. LOGRIECO,¹ A. MORETTI,¹ G. MULÈ¹ and G. RANDAZZO⁴

¹ C.N.R. Istituto tossine e micotossine da Parassiti vegetali, Bari.

² Dipartimento di Patologia vegetale, Università di Bari.

³ Dipartimento di Scienze Chimico-agrarie, Università «Federico II», Portici (Napoli).

⁴ Istituto di Industrie agrarie, Università «Federico II», Portici (Napoli).

Investigations on a strain of *Fusarium avenaceum* (Fr.) Sacc. (ITEM-620) from wheat kernels collected in Yugoslavia, producing highly toxic extracts to brine shrimp (*Artemia salina* L.) larvae when grown on maize kernels in laboratory, led to the isolation of enniatin B.

Enniatin B was purified in yields of 121 mg/Kg of culture by CC and preparative TLC, using brine shrimp bioassay to monitor the toxicity of the fractions, and characterized essentially by ¹D ¹H- and ¹³C-NMR and by EI-HRMS and FAB-MS. Moreover its physical properties resulted very consistent with the literature data. The 50% lethal dose of enniatin B on *A. salina* was calculated in 8.6 µg/ml of sea water.

These results prompt to extend the investigation on enniatin B production by 13 *F. avenaceum* isolates on different substrates (corn, wheat). Enniatin B was produced (20 to 167 mg/Kg) by 5 isolates and higher yields were obtained on wheat.

In considering its toxicity, it appears that enniatin B could represent serious problems especially for wheat crops.

TOXIC METABOLITES OF *PYRENOPHORA GRAMINEA*

M. ARAGONA,¹ A. PORTA-PUGLIA,² A. GATTI³ and G. VANNACCI³

¹ Istituto Sperimentale per la Patologia Vegetale, Roma.

² Istituto Sperimentale per la Cerealicoltura, Fioresnuola d'Arde.

³ Dipartimento di Coltivazione e Difesa delle Specie Legnose, Università di Pisa.

Leaf-stripe of barley (*Hordeum vulgare* L.), caused by *Pyrenophora graminea* Ito et Kuribayashi, occurs throughout the world and can induce serious yield losses. Until now the control of the pathogen, which is essentially seed-transmitted, has