ADVANCES ON THE TOXICITY OF THE CEREAL CONTAMINANT *FUSARIUM* ESADEPSIPEPTIDES

Antonio LOGRIECO[1] – *Antonio MORETTI*[1] – *Giuseppina MULE'*[1] – *Costantino PACIOLLA*[2] – *Alberto RITIENI*[3]

[1]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Amendola 122/O, 70125 Bari, Italy.
[2]Dipartimento di Biologia e Patologia Vegetale- Sezione di Biologia Vegetale- Università degli Studi di Bari, via Amendola 165/A, 70125 Bari, Italy.
[3]Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", via Università 100, Parco Gussone Edificio 84, Portici -Napoli, I-80055, Italy.

Abstract: *Fusarium* head blight (FHB) of cereals is a well known disease caused by a complex of several toxigenic species of *Fusarium*. FHB can reduce grain yield and quality, because of the accumulation of mycotoxins in cereal grains and derived foods and feeds. The pathogen mainly reported as causal agent of FHB is *F. graminearum*, that produces Deoxynivalenol (DON), the mycotoxin mostly associated to the disease. However in the last decade, in Europe, in addition to DON, the esadepsipeptides Enniatins (ENs) and Beauvericin (BEA) have been often reported as cereal contaminants, in association with different species such as *F. avenaceum*, *F. poae*, and *F. tricinctum*. The natural occurrence of high amounts of BEA and ENs in FHB small grains, evaluated with the phytotoxic and zootoxic properties of these metabolites, compel to an examination of their potential role in contributing to the severity of FHB. On the other hand, the recent studies that have provided further data on the biological role of the esadepsipeptide in plants and their toxicity toward plants, animal and humans, make it worthwhile to expand the knowledge on the significance and the toxicity of these frequent contaminants of cereals.

Keywords: *Fusarium* Head Blight (FHB), Esadepsipeptides. Enniatins. Beauvericin. Mycotoxins. *Fusarium avenaceum*.

Introduction

Fusarium head blight (FHB) of wheat and other small cereals is a severe disease worldwide, causing reduction in crop yield often estimated up to 30 percent. In addition many *Fusarium* species are also capable of producing mycotoxins which can be formed already in infected plants in the field and then accumulated in stored grains under favourable fungal growth conditions. The occurrence of mycotoxins in cereal grains is of great concern, because their presence in foods and feeds is often associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, also in human. The *Fusarium* species predominantly reported around the world as causal FHB agent is *F. graminearum*, and the toxicological connected greatest problem is related essentially with the occurrence of deoxynivalenol (DON). However, in the last decade in Europe, in addition to DON, the esadepsipeptides Enniatins (ENs) and Beauvericin (BEA) have been often reported as cereal contaminants, in association with different species such as *F. avenaceum*, *F. poae*, and *F. tricinctum* (Logrieco and Moretti 2008). Many strains of *F. avenaceum* from FHB small grains were found to produce several well known cyclic esadepsipeptides (Logrieco et al. 1998; Logrieco et al. 2002b; Morrison et al. 2002). In addition, some other *Fusarium* species from FHB grains, such as *F. poae*, and *F. tricinctum* proven to have the same relevance as esadepsipeptide producers (Langseth et al. 1999; Yli-Mattila et al. 2004a). On the other hand, there are increasing evidences of the occurrence of high amounts of ENs and BEA in Finnish scab small grains (wheat, rye, oats and barley) (Logrieco et al. 2002b; Jestoi et al. 2004a, Uhlig et al. 2006) and in

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Norwegian wheat (Uhlig et al. 2006). Enniatins were known for a long time as phytotoxins and associated with plant diseases characterized by wilt and necrosis (Gäumann et al. 1960), and some phytotoxic properties were reported also for BEA (Sagakuchi et al. 2000, Paciolla et al. 2004). Moreover, due to their ionophoric structure, BEA and ENs are able to exhibit many toxic effects on animal systems, starting from the alteration of the ion transport across membranes, which may lead to the disruption of the cationic selectivity of cell wall, and ultimately to induce DNA fragmentation and cell death by apoptosis (Dombrink-Kurtzman, 2003; Kouri et al. 2003; Kamyar et al. 2004). This basic mechanism, associated with the acyl-CoA:cholesterol acyltransferase (ACAT) inhibition (Tomoda et al. 1992b), represents the behaviour leading to a large array of toxic ability, such as antimicrobial (Fotso and Smith, 2003), and a strong cytotoxic activity on several cell lines of murine (Wu et al. 2002; Hoornstra et al. 2003; Iwata et al. 2003), farm animal (Morrison et al. 2002; Dombrink-Kurtzman, 2003; Uhlig et al. 2004), and human (Macchia et al. 2002; Calò et al. 2004; Ivanova et al. 2006; Lee et al. 2008). These finding on toxic potentiality of BEA and ENs in plant and animal systems, obviously stimulate us to a deeper examination of the significance of such esadepsipeptides and their producing *Fusarium* species in contributing to the FHB severity and grains toxicity.

Fusarium Head Blight (FHB) of cereal grains and natural occurrence of Beauvericin and Enniatins.

Symptoms and causal agents. The etiological characteristic of FHB is the co-occurrence, or the quick succession, of several *Fusarium* species usually referred as a "complex". Strains of the less-pathogenic or opportunistic *Fusarium* species also can produce considerable amounts of mycotoxins as *F. graminearum*, the pathogenic and predominant *Fusarium* species associated to FHB. Thus, the toxigenic profile of a contaminated crop is related not only by the predominant pathogenic *Fusarium* species, but also by the opportunistic species making up the "complex". Recent investigations reported an increasing diffusion of toxigenic strains of *F. avenaceum* as the most important agent of FHB of small cereal grain (wheat, barley, rye, and oats) in Northern European countries, including Norway (Langseth et al. 1999; Morrison et al. 2002; Kosiak et al. 2003) and Finland (Logrieco et al. 2002b; Yli-Mattila et al. 2004a). Besides their pathogenic ability, the spreading strains of *F. avenaceum* exhibited also a high capability to synthesize esadepsipeptides which led to presume a role of these metabolites in disease development and in grain toxicity (Morrison et al. 2002; Jestoi et al. 2004b). In relation to crop year and to cereal host, together with *F. avenaceum*, other *Fusarium* species are assuming a relative importance, including strains of *F. poae* and *F. tricinctum,* with a similar high capability to produce BEA and ENs. This shift of the FHB complex towards *Fusarium* species esadepsipeptide producer is a new characteristic of the small cereal scab mostly in northern European regions, and prompted many questions about the toxicological risks as well as on the etiological agents (Jestoi et al. 2004b; Yli-Mattila et al. 2004a). As a further species considered as esadepsipeptide producer, *F arthrosporioides* has been often reported as small cereal contaminant in Northern Europe (Yli-Mattila et al. 2004a). However, the taxonomic status of this species is still to be defined since recent phylogenetic analysis using *β*-

tubulin, IGS and ITS sequences did not clearly separate *F. arthrosporioides* from *F. avenaceum* (Yli-Mattila et al. 2004b). Finally, it is interesting to underline a recent disease of wheat, the black point disease, reported by Desjardins et al. (2007). The causal agent of the disease is *F. proliferatum*, a toxigenic pathogen of maize worldwide, known to produce fumonisin B_1 (FB_1). This species is also well known as the main responsible for BEA accumulation in maize together with *F. subglutinans* (Logrieco and Moretti 2008). Therefore, since in the wheat affected by kernel black point disease, the mycotoxin risk has been associated to FB_1, the occurrence of BEA also should be accurately evaluated. **Natural occurrence**. BEA is the main natural component of the BEA group, belonging to ENs family (Hamill et al. 1969). In addition to BEA, five other compounds were purified from fumgal cultures, and designated as beauvericin A, C, D, E and F (Gupta et al. 1995; Nilanonta et al. 2000; Tomoda et al. 1992a; Fukuda et al. 2004), but they are not yet found as natural contaminants. The most important ENs, reported as natural contaminants, include: Enniatin A (ENA), Enniatin A1 (ENA1); Enniatin B (ENB); and, Enniatin B1 (ENB1) (Savard and Blackwell, 1994). Beauvericin, even occasionally, has been detected all around the world in a variety of commodities, mainly in maize (ear rot and commercial kernels) infected by *F. subglutinans* and *F. proliferatum* (Desjardins 2006). With respect to small cereals, esadepsipeptides were common in Finnish and Norwegian wheat and other small cereal grains mainly referred to the infections of *F. avenaceum*), but also to those of *F. poae* and *F. sporotrichioides* ((Logrieco et al. 2002b; Yli-Mattila et al. 2004a; Jestoi et al. 2004a; Uhlig and Ivanova 2004; Uhlig et al. 2006). In particular, the toxins were found in all samples of Finnish rye (Logrieco et al. 2002a); in Norwegian samples of wheat, barley and oats (Uhlig et al. 2004; Uhlig et al. 2006); in Finnish samples of wheat, barley and oats (Jestoi et al. (2004a); and, in trace level in Italian and Finnish samples of grain-based products (Jestoi et al. 2004b).

Biological properties of Beauvericin and Enniatins.

The primary toxic action of BEA and ENs is considered to be derived from their ionophoric properties, which enable their molecules to form stable complexes with cations, and transport them into the lipophilic phase (Hilgenfeld and Saenger 1982). Besides these studies, which suggest that in the membrane BEA acts as an ion carrier, other observations showed that BEA, when incorporated in synthetic membrane or in mammalian cells, is able to forms a cation selective channel, constituted by a sandwich-structure with a molecular ratio 2:1 with cations, allowing the cation to span the membrane (Kouri et al. 2003). Moreover, it seems that the influence of BEA on physiological cell ion balance involve several ions, as indicated by the electromechanical and –physiological observations carried on the activity of BEA on isolated smooth and heart muscle preparations of the guinea pig, showing that this metabolite not only affects the calcium current, as suggested by the effects on contractility, but also interacts with the sodium inward and potassium outward currents (Lemmens-Gruber et al. 2000). A similar pattern for ion transporting through the membrane was proposed also for ENs. In particular, the ENs transport of the cations in liposome seems to involve a mobile carrier mechanism selective for K^+ versus Na^+, which requires two enniatin molecules, and the transport efficiency appears to be related

to the hydrophobic trait of the enniantin molecules and is realized by a "sandwich" model (Kamyar et al. 2004). **Apoptotic effect.** Beauvericin induces programmed cell death mediated by apoptosis, accompanied by internucleosomal DNA fragmentation into multiples of 180-200 base pair-fragments (Dombrink-Kurtzman 2003). The apoptotic effect of BEA seems a consequence of its ionophoric properties (Ojcius et al. 1991). This mechanism has been confirmed also by Holownia et al. (1997), that showed an increase in intracellular calcium and the subsequent activation of the calcium-dependent endonucleases leading to DNA fragmentation. To this regard, investigations carried out by Pocsfalvi et al. (1997) on the possible non-covalent adduct formations between DNA and oligonucleotides, showed that BEA is able to form more stable association complex with DNA than other fusariotoxin, such as fumonisin and fusaproliferin. For its high capability to induce apoptosis, BEA was used as a model in a number of investigations on cell physiopathology, employing bioassay on several specific cell lines, such as on: lymphocytes for studies on oncogenesis (Cook et al. 1999); cultured rat astrocytes for studies of the effects of ethanol on brain (Holownia et al. 1999); mouse neuroblastoma and rat glioma hybrid line (NG108-15) for observations on ion (Ca^{+2}) current in neuronal cell lines (Wu et al. 2002); human and rat cholangiocytes for studies on primary biliary cirrhosis (Harnois et al. 1997; Que et al. 1999); murine cultured biliary epithelial cells (BECs) in a dose- and incubation time-dependent manner (Iwata et al. 2003). Finally, Klaric et al. (2008) studied a porcine kidney epithelial cell line PK 15 and showed that BEA, by increasing the caspase-3 activity, caused significant morphological apoptotic changes in the PK 15 cells. Interestingly, the effects of BEA on PK 15 cells were analyzed together with FB_1 and ochratoxin A (OTA), and data showed that BEA and OTA had additives effects of on the lactate dehydrogenase activity, while they had additives and synergistic effects on caspase-3 activity and apoptotic index (Klaric et al. 2008). **General toxicity.** It is likely that the number of toxic effects reported for BEA and ENs, including cytotoxic, antimicrobial, insecticidal, and phytytotoxic activities, are the results of the above main toxic mechanism, connected with their ionophoric, apoptotic and ACAT activities (Tomoda et al. 1992a). Some of these consequential toxic effects are here reported in detail. **Cytotoxic activity.** The several observations on the cytotoxic acticity of BEA on murine cell lines, all recognized BEA as a strong cytotoxic agent. Evidences obtained include: the increase of intracellular Ca^{2+} and DNA fragmentation followed by apoptosis at concentration up to 50 μM in PO815, Yac-1 and EL-4 murine tumor cells (Ojcius et al. 1991), and at concentration of 10 μM in rat brain cells (Holownia et al. 1997); specific inhibition of ACAT in J774 mouse macrophages (IC_{50} = 11 μM) (Tomoda et al. 1992b); induction of apoptosis in several mouse cell lines (L-1210-Fas, A20, WEHI-231) at concentration of 10-20 μM (Nash et al. 1998); induction of apoptosis in a dose- and time-dependent action in mouse biliary epithelial cells (BEC) at concentration of 5-50 μM (Iwata et al. 2003). Then, the potential neuronal activity of BEA was demonstrated by Wu et al. (2002) in a mouse neuroblastoma and rat glioma hybrid cell line (NG108-15), suggesting that BEA is a relatively specific inhibitor of calcium current in such neuronal cell line. The cytotoxic potentiality of ENs on livestock cell lines was assayed on boar spermatozoa (Hoornstra et al. 2003), and on porcine kidney cells (PK15) (Uhlig et al. 2004). On boar spermatozoa, ENS caused mitochondrial membrane damage and a reduction of the cell motility; whereas on PK15

cells caused a decrease of the metabolic activity. The cytotoxicity of BEA was also tested toward a porcine kidney epithelial cell line PK 15 (Klaric et al. 2007), showing that BEA affected the viability of the cell line by decreasing the lipid peroxidation and intracellular glutathione activities. In this study, the effects of BEA on PK 15 cells were combined with those of FB_1 and OTA, and clearly showed additives effects of these three toxins (Klaric et al. 2007). The awareness that BEA was also an important toxic secondary metabolite of *Fusarium* strains colonizing cereals, with the possibility to contaminate food and feed, led to focus on cytotoxicity to human cells with a particular interest. The effect of BEA was examined by Macchia et al. (2002) in a wide array of human cell lines, including cell lines of emopoietic origin. In all these assays, the toxicity of BEA was dose- and time-dependent, and a substantial decrease of viability was seen after 4 h of incubation already. Moreover, BEA reduced the cell viability of human cholangiocytes cell line (H-69), inducing DNA fragmentation and apoptosis at (Que et al. 1999); and decreased the metabolic activity of human epidermoid carcinoma cell line (KB) and human breast cancer cell line (BC-1) (Nilanonta et al. 2002). The cytotoxicity of BEA was also assayed on human cell lines of myeloid origin, namely, monocytic lymphoma cells U-937, and the promyelocytic leukemia cells HL-60, and a decrease of metabolic activity determined as cytotoxic concentration (CC_{50}) was estimated at 24h in 30 μM and 15 μM for U-937 cells and HL-60 cells, respectively (Calò et al. 2004). Further studies showing that BEA induces death in human leukemia cells (CCRF-CEM) were performed by Jow et al. (2004). In this study, several well known characterized factors, known to play important role in apoptotic pathway, were investigated in BEA-induced cell death. The data revealed that BEA-induced cell death in CCRF-CEM cells was dose- and time-dependent and incidence of nuclear fragmentation and apoptotic body formation was significantly increased by BEA. Moreover, cytosolic capsase-3 activity and release of cytochrome c from mitochondria were increased by BEA, showing that these factors were related to the subsequent apoptotic cellular changes in morphology (Jow et al. 2004). Assayed on smooth and cardiac muscle of guinea pigs preparations, BEA reduced the contraction of terminal ilea in a concentration-dependently (IC_{50} = 18 μM), suggesting a potential cardiotoxic risk for this toxin (Lemmens-Gruber et al. 2000). A similar activity was reported by Kamyar et al. (2004) for ENs that, assayed on heart muscle preparations, on ventricular myocytes and terminal ilea of guinea pig, were able to reduce the duration of potential action and to decrease the force of contraction. Two cell lines of human origin, hepatocellular carcinoma-line and fibroblast-like foetal lung cell line, were studied by Ivanova et al. (2006) for the cytotoxicity of BEA and ENs (EN A, A1, B, B1, B2 and B3) together with DON. The data showed that the toxicity of the esadepsipeptides was comparable to DON and indicated these metaboltis may have an underestimated toxic potential. Lee et al. (2008) confirmed this hypothesis, by studyng the toxicity of En H, I, and MK 1688 toward four human carcinoma lines that resulted highly capable to inhibit the growth of all cell lines tested. **Phytotoxic activity**. Enniatins were supposed to involved in wilting and necrosis mechanisms of plants affected by *Fusarium* and, assayed on Tomato cuttings, reproduced some of the natural symptoms (loss of turgor, leaf yellowing and marginal necrosis), with ENB more toxic (21 mg/mg) than ENA (36 mg/mg), but with a synergistic action of ENB+ENA (6.8 mg/mg) (Gäumann et al. 1960). Other reports on phytotoxic activities of ENF include: inhibition of growth of

wheat seedlings (reduced growth of shoots and rootlets) (ENs mixture at 10-80 µg/ml) (Burmeister and Plattner 1987); formation of necrotic lesions on leaves of spotted knapweed (*Centaurea maculosa*) (22 µg of ENB) (Hershenhorn et al. 1992) and on potato tuber slices (ENs mixture at 10-100 µg) (Herrmann et al. 1996); and inhibition of parasitic weed (*Striga hermontica*) germination (50% with ENA 10^{-4}) (Zonno and Vurro 1999). Informations on the toxicity of BEA against plant systems are very limited. Preliminary studies reported that BEA was highly toxic to melon protoplasts compared with fusaric acid and fumonisin B_1 (Sagakuchi et al. 2000). Further observations carried out in comparison with T-2 toxin, a well known toxin produced by phytopathogenic strains of *Fusarium*, showed that both toxins induce premature protoplast death, but the phytotoxic action of BEA was much more severe than that of T-2, in terms of reduction in protoplast viability. The reduction of the ascorbate peroxidase activity after BEA treatment, not observed for T-2 toxin, suggested a possible distinctive role of the ascorbate system in tomato protoplast death caused by BEA (Paciolla et al. 2004). In addition, BEA showed a different trend of antioxidant defence responses than T-2 mycotoxin towards tomato plants. BEA stimulated simultaneous mobilization of different defence systems of tomato plants in order to overcome the BEA phytoxicity. Structural modifications of the cell wall, such as enhanced lignification, and metabolic rearrangement brought about by intracellular antioxidant systems are suggested to decrease the ionophore effect of BEA in tomato plants (Paciolla et al. 2008). **Pharmacological properties.** In traditional Chinese medicine, BEA is used for its ACAT activity to lower the cholesterol levels of blood, and a patent has been issued for the use of BEA in tablet containing 5 mg of pure compound. Moreover, BEA is also used as a constituent in antineoplastic and anticonvulsant medicines (Huang et al. 1999). Interestingly, BEA has been found to be a chemosensitizing agent, acting as a potentiator of antifungal miconazole activity and thus promoting the antibiotic effectiveness (Lee et al. 2001; Fukuda et al. 2004); and was reported to have some possibility for a clinical use in combination with chemotherapic drug in human cancer therapy (Sharom et al. 1998). Finally, due to their antibiotic properties ENs, in a mixture called fusafungin (ENA+ENA1+ENB+ ENB1, 2:16:40:42), are employed as an oral or nasal inhalation solution (1%) against diseases of the upper respiratory tract (Kroslák 2002). Finally, of extreme importance is the study carried by Zhang et al. (2007) that showed the capability of BEA to act as synergistic compound combined with ketoconazole (KTC), a weak antifungal molecule used to control fungal infection in immunocompromised patients. The authors investigated the effects of BEA and KTC together in order to reduce the infection of the hosts infected by *Candida parapsilosis* and showed that BEA increased more than 100 times the capability of KTC of prolonging the survival of patients by reducing the colony counts of Candida *parapsilosis* in animal organs such as kidneys, lungs, and brains (Zhang et al. 2007). These data underline the extreme importance of the biological role of BEA and open further challenging field of investigation on this metabolite.

References

Burmeister, H.R., Plattner, R.D. 1987. Enniatin production by *Fusarium tricinctum* and its effect on germinating wheat seeds. Phytopathology **77**:1483-1487.

Calò, L., Fornelli, F., Ramires, R., Nenna, S., Tursi, A., Caiaffa, M.F., Macchia, L. 2004. Cytotoxic effects of the mycotoxin beauvericin to human cell lines of myeloid origin. Pharmacological Research **49**:73-77.

Cook, J.L., Routes, B.A., Leu, C.Y., Walker, T.A., Colvin, K.L. 1999. E1A oncogene-induced cellular sensitization to immune-mediated apoptosis in independent of *p53* and resistant to blockade by E1B19 kDa protein. Experimental Cell Research, **252**: 199-210.

Desjardins, A.E. 2006. *Fusarium* mycotoxins: Chemistry, Genetics and Biology. American Phythopathological Society Press. St. Paul, Minnesota, USA.

Desjardins, A.E., Busman, M., Proctor, R.H., Stessman, R. 2007. Wheat kernel black point and fumonisin contamination by Fusarium proliferatum. Food Additives and Contaminants **24**:1131-1137.

Dombrink-Kurtzman, M.A., 2003. Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes. Mycopathologia, **156:**357-364.

Fotso, J., Smith, J.S. 2003. Evaluation of beauvericin toxicity with the bacterial bioluminescence assay and the Ames mutagenicity bioassay. Journal of Food Science **68**:1938-1941.

Fukuda, T., Arai, M., Yamaguchi, Y., Masuma, R., Tomoda, H., Omura, S. 2004. New beauvericins, potentiators of antifungal miconazole activity, produced by *Beauveria* sp. FK1-1366. Taxonomy, fermentation, isolation and biological properties. Journal of Antibiotics (Tokyo) **57**:110-116.

Gäumann, E., Naef-Roth, St., Kern, H. 1960. Zur phytotoxischen Wirksamkeit der Enniatine. Phytopathologische Zeitschrift **40**:45-51.

Gupta S., Montllor, C., Hwang, Y.S. 1995. Isolation of novel beauvericin analogues from the fungus *Beauveria bassiana*. Journal of Natural Products **58**:733-738.

Hamill, R.L., Higgens, C.E., Boaz, H.E., Gorman, M. 1969. The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*. Tetrahedron Letters **49**:4255-4258.

Harnois, D.M., Que, F.G., Celli, A., LaRusso, N.F., Gores, G.J. 1997. Bcl-2 is overexpressed and alters the threshold for apoptosis in a cholangiocarcinoma cell line. Hepatology **26**:884-890.

Herrmann, M., Zocher, R., Haese, A. 1996. Enniatin production by *Fusarium* strains and its effect on potato tuber tissue. Applied and Environmental Microbiology **62**:393-398.

Hershenhorn, J., Park, S.H., Stierle, A., Strobel, G.A. 1992. *Fusarium avenaceum* as a novel pathogen of spotted knapweed and its phytotoins acetamido-butenolide and enniatin B. Plant Science **86**:155-160.

Hilgenfeld, R., Saenger, W. 1982. Structural chemistry of natural and synthetic ionophores and their complexes with cations. In: Boschke, F.L. (ed.),Topic in Current Chemistry Springer-Verlag, Berlin, Vol. 101, p. 1-82.

Holownia, A., Ledig, M., Menez, J.F. 1997. Ethanol-induced cell death in cultured rat astroglia. Neurotoxicology and Teratology **19**:141-146.

Holownia, A., Ledig, M., Braszko, J.J., Menez, J.F. 1999. Aceraldehyde cytotoxicity in cultured rat astrocytes. Brain Research **833**:202-208.

Hoornstra, D., Andersson, M.A., Mikkola, R., Salkinoja-Salonen, M.S. 2003. A new method for *in vitro* detection of microbially produced mitochondrial toxins. Toxicology in Vitro, **17**:745-751.

Huang, Y.G., Barl, B., Ivanochko, G. 1999. Selected non-timber forest products with medicinal application from Jilin province in China. In: Davidson-Hunt, I., Duchesne, L.C., Zasada, J.C. (eds), Forest communities in the third millennium: linking research, business and policy toward a sustainable non-timber forest product sector. Proceeding of the Ontario Meeting, October 1-4, 1999. Kenora, Ontario, Canada, 000-000.

Ivanova L., Skjerve, E., Eriksen, G.S., Uhlig, S. 2006. Cytotoxicity of enniatins A, A1, B, B1, B2, and B3 from Fusarium avenaceum. Toxicon, **47**:*868-876*.

Iwata, M., Harada, K., Katayanagi, K., Saito, T., Kaneko, S., Lobayashi, K., Nakanuma, Y. 2003. Apoptosis of murine cultured epithelial cells induced by glicochenodeoxycholic acid involves Fas receptor and its ligand. Hepatology Research **25**:329-342.

Jestoi, M., Rokka, M., Yli-Mattila, T., Parikka, P., Rizzo, A., Ritieni, A., Peltonen, K. 2004a. Presence and concentrations of the *Fusarium*-related mycotoxins beauvericin, enniatins and moniliformin in Finnish grain samples. Food Additives and Contaminants **21:**794-802.

Jestoi, M., Somma, M.C., Kouva, M., Veijalainen, P., Rizzo, A., Ritieni, A., Peltonen, K. 2004b. Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets. Molecular and Nutritional Food Research **48**:000-000.

Jow, G.M., Chou, C.J., Chen, B.F., Tsai, J.H. 2004. Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome c release, caspase 3 activation: the causative role of calcium. Cancer Letters **216**:165-173.

Kamyar, M., Rawnduzi, P., Studenik, C.R., Kouri, K., Lemmens-Gruber, R. 2004. Investigation of the electrophysiological properties of enniatins. Archives of Biochemistry and Biophysics **429**:215-223.

Klaric, M.S., Rumora, L., Ljubanovic, D., Pepeljnjak, S. 2008. Cytotoxicity and apoptosis induced by fumonisin B_1, in porcine kidney PK 15 cells: effects of individual and combined treatment. Archives of Toxicology **82**:247-255.

Klaric, M.S., Pepeljnjak, S., Domijan, A.M., Petrik, J. 2007. Lipid peroxidation and glutathione levels in porcine kidney PK 15 cells after individual and combined treatment with fumonisin B_1, beauvericin and ochratoxin A. Basic Clinical Pharmacological Toxicology **100**:157-164.

Kosiak, B., Torp, M., Skjerve, E., Thrane, U. 2003. The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. Acta Agriculturae Scandinavica, Section B: Soil and Plant Science **53**:168-176.

Kouri, K.M., Lemmens, M., Lemmens-Gruber, R. 2003. Beauvericin-induced channels in ventricular myocytes and liposomes. Biochimica et Biophysica Acta (BBA)-Biomembranes **1609**:203-210.

Kroslak, M. 2002. Efficacy and acceptability of fusafungine, a local treatment for both nose and throat infections, in adult patients with upper respiratory tract infections. Current Medical Research and Opinion **18**:194-200.

Langseth, W., Bernhoft, A., Rundberget, T., Kosiak, B., Gareis, M. 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia **144**:103-113.

Lee, H.S., Song, H.H., Jeong, J.H., Shin, C.G., Choi, S.U., Lee, C. 2008. Citotoxicity of enniatins H, I, and MK 1688 from Fusarium oxysporum KFCC 11363P. Toxicon, **51**:1178-1185.

Lee, M.D., Calazzo, J.L., Staley, A.J., Lee, J.C., Warren, M.S., Fuernkranz, H., Chamberland, S., Lomocskaya, O., Miller, G.H. 2001. Microbial fermentation-derived inhibitors of efflux-pump-mediated drug resistance. Il Farmaco **56**:81-85.

Lemmens-Gruber, R., Rachoy, B., Steininger, E., Kouri, K., Saleh, P., Krska, R., Josephs, R., Lemmens, M. 2000. The effect of the *Fusarium* metabolites beauvericin on electromechanical and –physiological properties in isolated smooth and hearth muscle preparations of guinea pigs. Mycopathologia **149**:5-12.

Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A., Chelkowki, J. 1998. Beauvericin production by *Fusarium* species. Applied and Environmental Microbiology 64:3084-3088.

Logrieco, A., Moretti, A., Ritieni, A., Caiffa, M.F., Macchia, L. 2002a. Beauvericin: Chemistry, Biology and Significance. In: Upadhyay, R.K. (ed.), Advances in microbial toxin research and its biotechnological exploitation. Kluwer Academic/Plenum Publishers,. New York, p. 23-30.

Logrieco, A., Rizzo, A., Ferracane, R., Ritieni, A. 2002b. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. Applied and Environmental Microbiology **68**:82-85.

Logrieco A.F., and A. Moretti. 2008. Between emerging and historical problems: an overview of the main toxigenic fungi and mycotoxins concerns in Europe. *In: Mycotoxins*. (Leslie J., R. Bandyopadhyay, A. Visconti eds). www.cabi.org, 139-155.

Macchia, L., Caiffa, M.F., Fornelli, F., Calò, L., Nenna, S., Moretti, A., Logrieco, A., Tursi, A. 2002. Apoptosis induced by the *Fusarium* mycotoxin beauvericin in mammalian cells. Journal of Applied Genetics **43a**:363-371.

Morrison, E., Kosiak, B., Ritieni, A., Aastveit, A.H., Uhlig, S., Bernhoft, A. 2002. Mycotoxin production by *Fusarium avenaceum* strains isolated from Norwegian grain and the cytotoxicity of rice culture extracts to porcine kidney epithelial cells. Journal of Agricultural and Food Chemistry ***50***:3070-3075.

Nash, P.B., Purner, M.B., Leon, R.P., Clarke, P., Duke, R.C., Curiel, T.J. 1998. *Toxoplasma gondii*-infected cells are resistant to multiple inducers of apoptosis. Journal of Immunology ***160***:1824-1830.

Nilanonta, C., Isaka, M., Kittakoop, P., Trakulnaleamsai, S., Tanticharoen, M., Thebtaranonth, Y. 2002. Precursor-directed biosynthesis of beauvericin analogs by the the insect pathogenic fungus *Paecilomyces tenuipes* BCC 1614. Tetrahedron **58**: 3355-3360.

Ojcius, D.M., Zychilinsky, A., Zheng, L.M., Young, D.E. 1991. Ionophore induced apoptosis: role of DNA fragmentation and calcium fluxes. Experimental Cell Research **197**:43-49.

Paciolla, C., Dipierro, N., Mulè, G., Logrieco, A., Dipierro, S. 2004. The mycotoxins beauvericin and T-2 induce cell death and alteration in the ascorbate metabolism in tomato protoplasts. Physiological and Molecular Plant Pathology **65**:49-56.

Paciolla, C., Ippolito, M.P., Logrieco, A., Dipierro, N., Mulè, G., Dipierro, S. 2008. A different trend of antioxidant responses makes tomato plants less susceptible to beauvericin than T—2 mycotoxin phytotoxicity. Physiological and Molecular Plant Pathology (in press).

Pocsfalvi, G., Di Landa, G., Ferranti, P., Ritieni, A., Randazzo, G., Malorni, A. 1997. Observation of non-covalent interaction between beauvericin and oligonucleotides using rlrctrospray ionization mass spectrometry. Rapid Communication in Mass Spectrometry **11**:265-272.

Que, F.G., Phan, V.A., Phan, V.H., LaRusso, N.F., Gores, G.J. 1999. GUDC inhibits cytochrome c release from human cholangiocyte mitochondria. Journal of Surgical Research **83**:100-105.

Sagakuchi, M., Moretti, A., Endo, E., Matsuda, Y., Toyoda, H., Ouchi, S. 2000. An approach to the use of plant sensitivity for simple detection of mycotoxins. In: Proceeding of First Asian Conference of Plant Pathology. Kuala Lumpur, Malaysia, August 2000, 262-279.

Savard, M.E., Blackwell, B.A. 1994. Spectral characteristics of secondary metabolites from *Fusarium* fungi. In: Miller, J.D., Trenholm, H.L. (eds), Mycotoxins in grain, compounds other than aflatoxin. Eagan Press, St.Paul, Minnesota, USA.

Sharom, F.J., Lu, P., Liu, R., Yu, X. 1998. Linear and cyclic peptides as substrates and modulators of P-glycoprotein: peptide binding and effects on drug transport and accumulation. Biochemical Journal **333**:621-630.

Tomoda, H., Nishida, H., Huang, X.H., Masuma, R., Kim, Y.K., Omura, S. 1992a. New cyclodepsipeptides, enniatin D, E and F produced by *Fusarium* sp. FO-1305. Journal of Antibiotics (Tokyo) **45**:1207-1215.

Tomoda, H., Huang, X.H., Cao, J., Nishida, H., Nagao, R., Okuda, S., Tanaka, H., Omura, S., Arai, H., Inoue, K. 1992b. Inhibition of acyl-CoA:cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. Journal of Antibiotics (Tokyo) **45**:1626-1632.

Uhlig, S., Ivanova, L. 2004. Determination of beauvericin and four other enniatins in grain by liquid chromatography-mass spectrometry. Journal of Chromatography A, **1050**:173-78.

Uhlig, S., Gutleb, A., Thrane U., Flåøyen, A. 2004. Identification of cytotoxic principles of *Fusarium avenaceum*. Poster presentation at the XI International IUPAC Symposium on Mycotoxins and Phycotoxins. Bethesda, May 2004, Maryland, USA.

Uhlig, S., Torp, M., Heier, B.T. 2006 Beauvericin and enniatins A, A_1, B and B_1 in Norwegian grain: A survey. Food Chemistry 94:193-201.

Wu, S.N., Chen, H., Liu, Y.C., Chiang, H.T. 2002. Block of L-type Ca^{2+} current by beauvericin, a toxic cylopeptide, in the NG108-15 neuronal cell line. Chemical Research in Toxicology **15**:854-860.

Yli-Mattila, T., Paavanen-Huhtala, S., Parikka, P., Hietaniemi, V., Jestoi, M., Rizzo, A. 2004a. Toxigenic fungi and mycotoxins in Finnish cereals. In: Logrieco, A., Visconti, A. (eds), An overview on toxigenic fungi and mycotoxins in Europe. Kluver Academic Publishers, The Netherlands, p. 83-100.

Yli-Mattila T., Paavanen-Huhtala, S., Parikka, P., Kostantinova, P., Gagkaeva, T.Y. 2004b. Molecular and morphological diversity of *Fusarium* species in Finland and north-western Russia. European Journal of Plant Pathology **110**:573-585.

Zhang, L., Yan, K., Zhang, Y., Huang, R., Bian, J., Zheng, C., Sun, H., Chen, Z., Sun, N., An, R., Min, F., Zhao, W., Zhuo, Y., You, J., Song, Y., Yu, Z., Liu, Z., Yang, K., Gao, H., Dai, H., Zhang, X., Wang, J., Fu, C., Pei, G., Liu, J., Zhang, S., Goodfellow, M., Jiang, Y., Kuai, J., Zhou, G., Chen, X. 2007. High-throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. Proceedings of the National Academy of Sciences of the United States of America **104**:4606-4611.

Zonno, M.C., Vurro, M. 1999. Effect of fungal toxins on germination of *Striga hermontica* seeds. Weed Research **39**:15-20.