Chapter 15
Occurrence and Physiology of Zearalenone as a New Plant Hormone

Jolanta Biesaga-Kościelniak and Maria Filek

Abstract Zearalenone* is a non-steroidal mycotoxin with oestrogenic properties, which is produced mainly by fungi belonging to *Fusarium* (*6-(10-hydroxy-6-oxo-trans–1–undecenyl)-β-resorcylic acid lactone). The toxin-producing ability of *Fusaria* is greatly influenced by environmental factors. Therefore, it was expected that the different weather conditions occurring during the vegetation period would be associated with differences in the preharvest occurrence of *Fusarium* toxins. Sustainable food systems research and practice concentrate on the study of the level of these mycotoxins in soils and crops. However, some experiments show that zearalenone can also act as a hormonal substance and have a favourable effect on the development of plants and animals. This chapter gives an overview of the possible effect of low concentrations of zearalenone on some physiological processes in crops. It has been shown that exogenous application of zearalenone and its derivatives can stimulate generative development in winter plants, which suggest its participation in the mechanism of flowering. Moreover, treatment with zearalenone had an effect on calli proliferation and cell differentiation. The effect of zearalenone was similar to the activity of auxins in *in vitro* cultures, which may confirm the hormonal properties of zearalenone in plants. Watering and soaking wheat and soybean grains with zearalenone solution resulted in higher yields of these plants. These observations, compared with the possibility of weather-related changes in the exogenous content of zearalenone in soils, can be useful in determining the optimal zearalenone dose that would show the favourable effect of this substance in plant development.

J. Biesaga-Kościelniak (✉) and M. Filek
Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology,
Niezapominajek 21, 30-239, Krakow, Poland
e-mail: J.Koscielniak@ifr-pan.krakow.pl

M. Filek
Institute of Biology, Pedagogical University, Podbrzezie 3,
3, 31-054, Kraków, Poland
and
Polish Academy of Sciences,
The Franciszek Górski Institute of Plant Physiology,
Niezapominajek 21, 30-239, Kraków, Poland

E. Lichtfouse (ed.), *Sociology, Organic Farming, Climate Change and Soil Science*,
Sustainable Agriculture Reviews 3, DOI 10.1007/978-90-481-3333-8_15,

Keywords Plant development • Plant hormones • Zearalenone

15.1 Introduction

Zearalenone is a mycotoxin produced by several *Fusarium* species. The term mycotoxin refers to a large number of chemically diverse toxic secondary metabolites formed by fungi imperfectly growing on agricultural commodities. Since the discovery of the aflatoxins in 1960 and subsequent recognition that mycotoxins are of significant health concern to both humans and animals, regulations gradually developed for mycotoxins in food and feed. *Fusarium* diseases of wheat, barley, and maize cause significant yield losses worldwide and are therefore of great economic importance (Sutton 1982; Diekman and Green 1992; Parry et al. 1995; Miedaner 1997; Mesterhazy et al. 1999; Malekinejad et al. 2007). The influence of host cultivars on the pathogenicity and toxicity of *Fusarium* fungi has been extensively reviewed (Miedaner 1997; Mesterhazy et al. 1999; Miedaner et al. 2001; Magg et al. 2002). Mycotoxins can contaminate grains in the field when environmental conditions favour fungal infection, and levels can increase dramatically if storage conditions are favourable for fungal growth. The influence of climatic factors on *Fusarium* diseases is complicated by the fact that *Fusarium* fungi can cause disease individually or in complex infections (Doohan et al. 1998), and there are numerous reports on how species differentially respond to different environmental variations, particularly temperature and humidity (Doohan et al. 2003). Therefore, it was expected that the different climatic conditions during the years surveyed would be associated with differences in the preharvest occurrence of *Fusarium* toxins. The European Commission has recently specified the maximum levels of *Fusarium* toxins that will be allowed from July 2006 onwards. Maximum levels of 200 and 100 μg/kg have been specified for zearalenone in unprocessed corn and unprocessed cereals other than corn, respectively (Javier et al. 2007; Hans et al. 2007)

In spite of the fact that contamination of cereals and grains and related products with mycotoxins causes food and feed-borne intoxications in man and livestock, zearalenone in low concentrations can be treated as a plant hormone which influences the development and yield of crop plants (Biesaga-Kościelniak 2001). This review focuses on the effect of low doses of zearalenone on the stimulation of selected physiological processes in plants important for agriculture production.

15.2 Chemical Structure of Zearalenone

Zearalenone, 6-(10-hydroxy-6-oxo-trans–1–undecenyl)-β-resorcylic acid lactone, is a non-steroidal mycotoxin with oestrogenic properties. It was first isolated from extracts of fungus *Gibberella zeae* (*Fusarium graminearum*) by Stob et al. (1962). This component is believed to act as an endogenous regulator of the sexual stage of development of their producer fungi. In the organisms of warm-blooded animals, the

lactones mimic endogenous 17 β-estradiol, i.e. they stimulate the growth of muscle tissue and affect the functions of the reproductive system (Burkin et al. 2002). Its chemical structure was determined by Urry et al. (1966), and its name is derived from *G. zeae*, the name of the first studied organism that produces it; *r*esorcylic *a*cid *l*actone, the generic name for this group of natural products; *ene*, the standard suffix indicating the presence of the C-1′ to C-2′ double bond; and *one*, the standard suffix indicating the presence of the C-6′ ketone (Fig. 15.1). Nowadays zearalenone is produced commercially by fermentation (Hidy et al. 1977) for use in the manufacture of zeranol (zearalanol) by catalytic hydrogenation (Hodge et al. 1966). It is a secondary fungal metabolite produced by several species of *Fusarium*, mainly by *F. graminearum* and *F. culmorum*. These species are known to colonize maize, barley, oats, wheat and sorghum (Eppley et al. 1974; Mirocha et al. 1974; Jemmali et al. 1978; Bennett and Shotweli 1979; Farnworth and Neish 1980; Kuiper-Goodman et al. 1987; Kuiper et al. 1988; Tanaka et al. 1988; Bennett and Klich 2003) and tend to develop during prolonged cool, wet growing and harvest seasons in the temperate and warm regions of the world (Velluti et al. 2000). Of numerous zearalenone derivatives that can be produced by *Fusarium* spp., only *trans*-α-zearalenol has been found to occur naturally in cereal grains (Richardson et al. 1985). After consumption of zearalenone, the two stereoisomeric metabolites, α- and β-zearalenole (Fig. 15.1), are produced in mammals by reduction of the keto-group at C-6′. Another structurally similar compound is zearalanol (zeranol, Ralgro), which is synthetically produced from zearalenone and is used as a growth promoter in animals and has been banned in the European Union since 1985 (Hagler et al. 2001; Nsahlai et al. 2002). Zearalanol is distinguished from zearalenone by lack of a C-1′–C-2′ double bond. This substance can also be formed in vivo from zearalenone and α-zearalenole, which can be carried over from contaminated feed stuff to animals. Zearalenone and zearalenoles (α and β) act as estrogens because they can adopt a conformation which sufficiently resembles 17 β-estradiol and other natural estrogens to enable binding to the estrogen receptor (King et al. 1978; Miksicek 1994). The physiological effects of zearalanol are similar to those of zearalenone, but zearalanol is generally considered to produce estrogenic effects five to ten times greater than those of zearalenone (Schollenberger et al. 2006).

Owing to their frequent occurrence, zearalenone and zearalenoles are an important class of endocrine disrupters. Their estrogenic potential is comparable to that of the naturally occurring estrogens estrone and estriol and is several orders of magnitude higher than those of well-known environmental estrogens, e.g. organochlorine pesticides (Mirocha et al. 1971, 1974; Krska and Josephs 2001; Dai et al. 2004).

15.3 Chemical and Physical Properties

Zearalenone is a white crystalline compound, which exhibits blue-green fluorescence when excited by long-wavelength UV light (360 nm) and a more intense green fluorescence when excited by short-wavelength UV light (260 nm). In methanol,

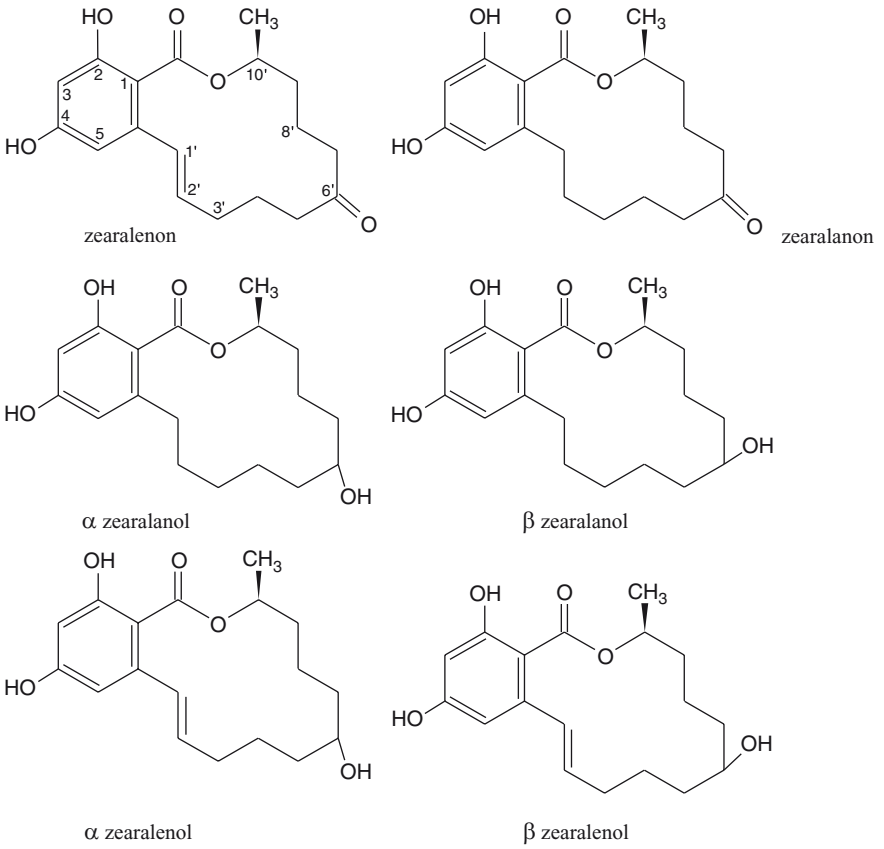

Fig. 15.1 The chemical structures of zearalenone and its derivatives

UV absorption maxima occur at 236, 274 and 316 nm. The molecular formula of zearalenone is $C_{18}H_{22}O_5$, its molecular weight is 318.4 g/mol and its melting point is 162–163°C (Blackwell et al. 1985; Josephs et al. 2003). The maximum fluorescence in ethanol occurs with irradiation at 314 nm and with emission at 450 nm. Its solubility in water is about 0.002 g/100 ml. In an aqueous solution of inositol, the presence of zearalenone can change the crystal structure of this alcohol, which indicates the possibility of interaction between both substances (our observations). Moreover, zearalenone is slightly soluble in hexane and progressively more so in benzene, acetonitrile, methylene chloride, methanol, ethanol and acetone. However, it is readily soluble in aqueous alkali.

In fungal cultures a number of closely related metabolites are formed, but there is only limited evidence that these occur in foodstuffs, although there is experimental evidence for some transmission of zearalenone and α- and β-zearalenols into the milk of sheep, cows and pigs fed with these substances at high concentrations. Zearalenone does not degrade at high temperatures (Zinedine et al. 2007), but may

be partly decomposed by heat. Approximately 60% of zearalenone remained unchanged in bread while about 50% survives in the production of noodles. Extrusion cooking may result in significant reduction of zearalenone with higher reductions of this substance at 120–140°C than at 160°C (Mateo et al. 2002).

15.4 Analytical Methods

Because estrogenic mycotoxins usually occur at microgram per kilogram (μg/kg) levels there is special interest in analytical procedures for reliable detection of zearalenone and its metabolites between 10 and 100 μg/kg. In response to the risk of a great economic loss to the industry and the threat to human health as a result of exposure to zearalenone, several methods have been developed for the quantification of zearalenone and its metabolites in different foods, feeds, animal tissues, blood and urine. Detailed reviews have been given by Steyn et al. 1991; Betina 1993; Frisvad and Thrane 1993; Scott 1993; Steyn 1995 and Lawrence and Scott 2000. The determination of zearalenone in cereals can be divided into five steps: grinding of the sample, extraction of the sample, clean-up, separation and detection.

In this regard several sophisticated chromatographic methods, with a quantification limit down to about 0.2 ng/g, have been developed and published for the determination of zearalenone. The methods were mainly based on high-performance liquid chromatography (HPLC) with fluorescence detection (Krska 1998; Visconti and Pascale 1998; Schuhmacher et al. 1998; Tanaka et al. 2000), but HPLC with mass spectrometry detection was also used (Shirai et al. 2000; Josephs et al. 2001).

Another method which uses capillary electrophoresis with laser-induced fluorescence detection can also be employed to detect zearalenone (Maragos and Appell 2007). In order to analyse trace amounts of zearalenone in plants, a sensitive, quick and accurate method, the enzyme-linked immunosorbent assay (ELISA) was developed by Chen et al. 1989.

15.5 Occurrence of Zearalenone in Plants

The occurrence of a zearalenone-like compound as a substance existing endogenously in plants was first reported by Li et al. (1980) and Li and Meng (1989). The aseptic culture of analysed shoot apices of the overwintering wheat plant confirmed that this substance was not due to fungal contamination, but was synthesized endogenously by the plants themselves. It was later confirmed by Meng et al. (1989) and Chen et al. (1989) using the enzyme-linked immunosorbent assay. This substance was identified by them as zearalenone.

The endogenous existence of zearalenone in plants served as a spur to further studies and its identification in different species. Han and Meng (1986) found zearalenone in rape, Meng et al. (1986) in winter wheat, Li and Meng (1989) in *Apium gaveoleus*, Que et al. (1990) in cotton, Han and Meng (1991) in *Lemna*

perpusilla and Fu and Meng (1994) in tobacco buds. Moreover, Meng et al. (1996) suggested the occurrence of this substance in more than 30 species of plants among others in onion, corn, rice, cotton, carrot, celery and apple. These data were not confirmed by other authors and the difficulty with their verification is connected with the fact that the majority of these articles are published in Chinese. However, our unpublished data indicate that small amounts of endogenous zearalenone can exist in winter wheat, soybean and spring rape. These measurements (high-performance liquid chromatography) were performed on plants cultured *in vitro* in sterile conditions.

15.6 Influence of Exogenous Zearalenone on Plant Generative Development

For agriculture plants, effective flowering is a very important process. In this process, a vegetative meristem changes into a reproductive meristem which is capable of forming floral organs and in this way completes the reproductive life cycle of higher plants (Bernier and Périllex 2005). How the vegetative meristem is able to perceive and interpret signals from the environment as well as from the plant itself is largely unknown. The process by which vernalization – the exposure of a germinating seed or a juvenile plant to a prolonged period of low temperature – promotes flowering in an adult plant has remained a mystery for many years (Michales and Amasimo 2000). Vernalization is an important control for many agricultural and horticultural production species in temperate regions.

Some studies indicated that exogenous zearalenone influences plant growth and development. For example, zearalenone stimulated the initiation of the vegetative bud in tobacco pith callus tissue (Mirocha et al. 1968), inhibited the cell membrane transport of maize roots (Vianello and Macri 1981) and enhanced the α-amylase and β-glucosidase activities of germinating maize seeds.

Meng et al. (1992) found that zearalenone was an endogenous regulator controlling induction of generative development in winter plant. An increase in endogenous zearalenone during vernalization was also recorded by Fu and Meng (1994) in many winter plants. Moreover, they suggested that exogenous zearalenone can partly replace the low temperature requirement for flowering in winter wheat.

In combination with greatly shortened vernalization (14 days, 5°C) zearalenone completely eliminated the flowering blockade of winter wheat cv. Grana, which usually requires vernalization of 8–9 weeks (Biesaga-Kościelniak 1998) (Table 15.1). Moreover, zearalenone in the concentration 2 mg/dm^3 reduced the length of the vegetative phase by as much as about 50 days in comparison with the control sample (Biesaga-Kościelniak 2001) (Fig. 15.2). The stimulating effect of zearalenone on the induction of heading was observed also in other wheat varieties, and its effectiveness was highest in those varieties which needed longer time of low temperature treatment to flowering induction (Table 15.2). Some zearalenone derivatives exercised a greater influence on the induction of heading and the rate of generative development

Table 15.1 The influence of zearalenone on the generative development of winter wheat cv. 'Grana' after various periods of vernalization (According to Biesaga-Kościelniak 1998, modified). Isolated wheat embryos were cultured in sterile conditions on Murashige and Skoog (1962) media supplemented with 0 (control), 0.25, 0.50, 0.75 and 2.00 mg/dm^3 of zearalenone during 14, 28 and 42 days at 5°C (vernalization). After these periods, plants were transferred to soil and cultivated at 20/17°C. Number of headed plants, generative development of apexes and number of vegetative ones was obtained after 100 days of grown at 20/17°C

	Number of plants			
Concentration of zearalenone (mg/dm^3)	Headed	Generative (non-headed)	Vegetative	Differentiation of frequency with respect to control
14 days of vernalization				
0.25	57 (95)[a]	3	0	+
0.50	13 (22)	47	0	+
0.75	57 (95)	3	0	+
2.00	60 (100)	0	0	+
0.00 (Control)	13 (22)	14	33	
28 days of vernalization				
0.25	59 (98)	1	0	+
0.50	57 (95)	3	0	+
0.75	60 (100)	0	0	+
2.00	60 (100)	0	0	+
0.00 (Control)	35 (58)	25	0	
42 days of vernalization				
0.25	59 (98)	1	0	
0.50	60 (100)	0	0	
0.75	60 (100)	0	0	
2.00	60 (100)	0	0	
0.00 (Control)	49 (83)	10	0	

'+'Significant differentiation on the basis of $\chi^2_{(p<0.01)}$ test
[a]In brackets percent of headed plants in population

of winter wheat cv. Grana than this substance itself (Table 15.3). Very strong activity has been demonstrated, in particular, by α-zearalanol, which after only 7 days of vernalization at 5°C induced the heading of almost all plants and greatly reduced the duration of the vegetative phase. The effectiveness of zearalenone was increased by an addition of spermidine and tissue extracts from inflorescences of some plant species. The effect of zearalenone on the growth process of wheat was to some extent contrary to its effect on the generative development, since it inhibited the elongation of the shoots, and also reduced their ability to accumulate biomass. The role of zearalenone in inducing flowering of winter wheat plants was confirmed by experiments with an exogenous application of a zearalenone synthesis inhibitor (malathion). This inhibitor decreased the plants' heading ability even after long vernalization (Table 15.4).

The influence of zearalenone on the generative development of winter rape was much weaker in comparison with that of wheat. None of the concentrations which stimulated wheat plants induced the flowering of rape plants. Zearalenone treatment stimulated only the first step of the process of the shoot apices generative differentiation (Biesaga-Kościelniak 2001).

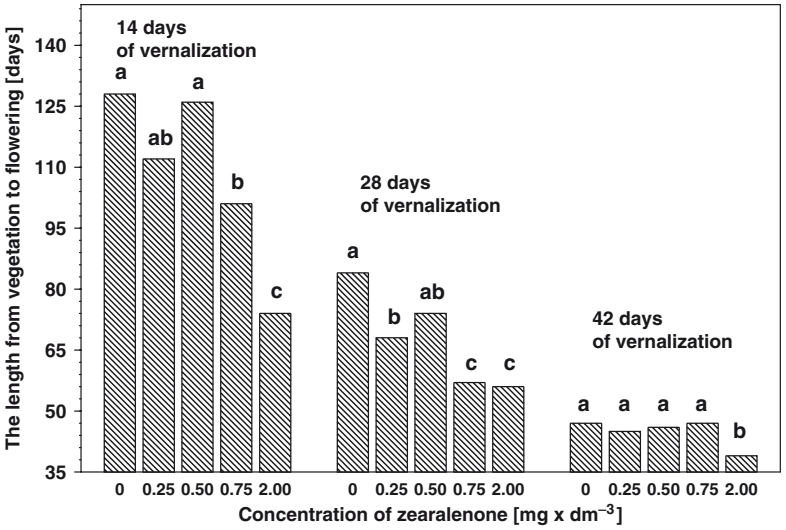

Fig. 15.2 The influence of zearalenone on the length of the phase from vernalization to flowering. Isolated embryos of winter wheat cv. 'Grana' were cultured in sterile conditions on Murashige and Skoog (1962) media supplemented with 0, 0.25, 0.50, 0.75 and 2.00 mg/dm^3 of zearalenone during 14, 28 and 42 days at 5°C (vernalization). After vernalization seedlings were transferred to soil and cultured at 20/17°C (day/night) to flowering. For particular length of vernalization values marked with the same letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$). For all investigated periods of vernalization, the best effect, observed as significant shortening of the length of the period between vernalization and flowering was noticed for 2 mg/dm^3 of zearalenone

Table 15.2 The influence of zearalenone on the generative development of nine winter wheat varieties. Isolated wheat embryos were cultured in sterile conditions on Murashige and Skoog (1962) media supplemented with 0 (control) and 2.00 mg/dm^3 of zearalenone during 14 days at 5°C (vernalization). After this period, plants were transferred to soil and cultivated at 20/17°C. The number of heading and vegetative plants was fixed after 100 days from vernalization. The length of the phase from vernalization to flowering was determined in days

	Number of plants				Differentiation of frequency with respect to control	Length of the phase from vernalization to flowering (days)	
	Control		Zearalenone				
Variety	Headed	Vegetative	Headed	Vegetative		Control	Zearalenone
Kaja	45 (90)[a]	5	44 (88)	6		62	55
Almari	40 (80)	10	39 (85)	7		75	77
Tercja	40 (80)	10	44 (83)	9		75	70
Maltanka	17 (35)	31	21 (42)	29		71	69
Wanda	18 (35)	33	25 (50)	25		81	82
Jubilatka	13 (26)	37	33 (65)	18	+	79	67*
Kamila	12 (25)	36	30 (60)	20	+	85	61*
Zorza	5 (10)	45	44 (83)	9	+	99	71*
Izolda	5 (10)	45	39 (85)	7	+	89	73*

'+' Significant differentiation on the basis of $\chi^2_{(p<0.01)}$ test
[a]In brackets percent of headed plants in population

Table 15.3 Generative development of winter wheat plants after treatment with derivatives of zearalenone (According to Biesaga-Kościelniak 1998, modified). Seeds of wheat cv. 'Grana' were cultured in sterile conditions on Murashige and Skoog (1962) media supplemented with zearalenone and its derivative solutions used in concentration 2 mg/dm^3 during 7 days at 5°C (vernalization). After vernalization plants were replaced to soil and cultured at 20/17°C. The number of heated and vegetative plants was fixed after 100 days of growth at 20/17°C, and for generative plants, the length of phase from vernalization to flowering was determined

Zearalenopne derivatives	Number of plants		Differentiation of frequency with respect to zearalenone	Length of phase from vernalization to flowering (days)
	Headed	Vegetative		
α-Zearalenol	30 (60)[a]	20		63 ab
β-Zearalenol	15 (30)	35		69 ab
Zearalanon	17 (35)	32		73 a
α-Zearalanol	49 (98)	1	+	47 c
β-Zearalanol	45 (90)	5	+	50 c
Zearalenone	30 (60)	20		65 b

'+' Significant differentiation on the basis of $\chi^2_{(p<0.01)}$ test
Mean values marked with the same letter in the last column do not differ significantly according to Duncan's multiple range test ($p < 0.05$)
[a]In brackets percent of headed plants in population

Table 15.4 The influence of the inhibitor of zearalenone (malathion) on the generative development of winter wheat plants. Seeds of wheat cv. 'Grana' were cultured at sterile condition on Murashige and Skoog (1962) media supplemented with 0 (control), melathion (10 ml/dm^3) and zearalenone (2 mg/dm^3) during 5 weeks at 5°C (optimal time of vernalization). After vernalization plants were replaced to soil and grown at 20/17°C. Percentage of headed plants and the length of phase from vernalization to flowering was determined for 100 plants in each kind of medium

Medium	Percent of headed plants	Length of phase from vernalization to flowering (days)
Malathion	16	74
Zearalenon	100	57
0 (Control)	72	77

Biochemical analysis indicated that the stimulation of the generative differentiation in wheat shoot apices after short vernalization, but in the presence of zearalenone was connected with an intensified emission of heat and a decrease in the value of the electric potential of the cells (Biesaga-Kościelniak 2001). Additionally, during vernalization of these plants and after vernalization, zearalenone induced changes in the composition of fatty acids in the fractions of membrane glycolipids and phospholipids. Zearalenone treatment resulted in the increase in content unsaturated fatty acids (calculated as 18:3 to 18:2 ratio). Such an increase in fatty acid unsaturation is usually a result of changes in cell membranes being exposed to low temperatures. On the other hand, zearalenone somewhat hampered the adjustment of the fluidity of the cell membranes, which was indicated by an increase in the content of campesterol and cholesterol in the seedlings. The observed dual effect of this substance on membrane composition is that it can stabilize membrane structure at low temperature, which allows specific domains located on membranes to become more prominent.

Such changes may be involved in the pathway of induction of generative development of winter plants induced by vernalization. The involvement of zearalenone in the vernalization process was suggested by Meng et al. (1996) who indicated two specific zearalenone-binding proteins (39.8 and 12.5 kDa) in the vernalized embryos of winter wheat. They postulated that these proteins might act as activators of certain genes controlling the vernalization process in plants.

The role of zearalenone in generative induction was also confirmed in photoperiodic plants (Meng et al. 1992, 1996; Fu et al. 1995, 2000), which suggests its importance in flowering stimulation. In the short-day plant *L. perpusilla 6746* and the long-day plant *L. gibba* G3, zearalenone enhanced flowering. In the day-neutral tobacco (*Nicotiana tabacum* L. cv. Samsun), zearalenone was one of the important flower stimuli and was related to the flower gradient in shoots (Meng et al. 1996). In the studies of Fu et al. (2000) a connection between zearalenone and flower bud formation in thin-cell layer explants of *N. tabacum* L was indicated. During the formation of flower buds, the authors observed two peaks in the endogenous zearalenone level, one at day 3 and the other at day 9 after the outset of the culture. The inhibitor of zearalenone biosynthesis (malathion), inhibited the biosynthesis of endogenous zearalenone and at the same time flower bud neoformation. Exogenous zearalenone application reduced the effect of malathion and stimulated flower bud neoformation.

15.7 The Effect of Zearalenone in Culture *In Vitro*

The presence of hormones (auxins and cytokinins or substances of similar action) is required for the induction, proliferation and differentiation of cells in *in vitro* cultures (Maheshwari et al. 1995). The dynamic development and the introduction of in vitro techniques to micropropagation and to the study of mechanisms of physiological processes have resulted in the need for the search for new groups of substances playing a role similar to those of plant hormones. Fusicoccine, cotynine, helmintosporine, pestalocine and some other metabolites isolated from fungi belong to this group (Muller et al. 1991).

In tissue culture of wheat and rape, the influence of zearalenone greatly resembled that of 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic analogue of auxin (Biesaga-Kościelniak 2001; Biesaga-Kościelniak et al. 2003). Zearalenone completely replaced 2,4-D or increased its effect under *in vitro* conditions. It increased the percentage of wheat calli capable of regenerating shoots by more than 2,4-D, and especially the process of effectively regenerating shoots from poorly differentiated wheat calli. Zearalenone enabled the breaking of the blockade of the regeneration of shoots from callus of winter rape cv. “Górczański”. Additionally, the application of thidiazurone to theses media increased the percentage of plant regenerated from callus for both wheat and rape. Therefore, it is possible to use zearalenone as an alternative to auxin or as a supplementary hormone analogue in *in vitro* culture of plants. This could be especially important when indirect regeneration of plants via callus induction is planned (Biesaga-Kościelniak et al. 2003; Szechyńska-Hebda et al. 2007).

Moreover, zearalenone stimulated the growth of cell suspension of winter wheat and winter rape (in aqueous media) by more than 2,4-D, contributing to the increment in the volume and dry weight of cells during the culture period (Biesaga-Kościelniak 2001). In the suspension culture of wheat, the addition of zearalenone to a medium containing 2,4-D caused not only an increase in the dry weight of cells, but also an increase in the population of living cells in the culture.

The maize pollination system was used as a model to compare the activity of zearalenone with 2,4-D (Biesaga-Kościelniak 1998). Zearalenone in a concentration 50 times lower than that of 2,4-D demonstrated a similar effectiveness in stimulating the development of haploid embryos in wheat flowers after pollination with maize pollen (Biesaga-Kościelniak et al. 2003). The concentration of zearalenone (6 mM) was most effective in inducing ovary swelling (84 swollen ovaries/100 pollinated florets) and increasing the frequency of embryo induction (18.9 embryos/100 pollinated florets), but these embryos were severely deformed. They had low capability to germinate *in vitro*, while callus was easily formed and indirect regeneration of plants was possible. The results showed that zearalenone had some of the properties of an auxin analogue, while other effects of its actions were unique.

Zearalenone was also found to be more effective than cytokinin treatment in inducing shoots in *in vitro* winter wheat production. Moreover, both zearalenone and cytokinins increased the activity of antioxidant enzymes in wheat callus undergoing regeneration, and it is very likely that they also stimulated the plant regeneration process (Szechyńska-Hebda et al. 2007). The effectiveness of regeneration on media containing zearalenone shows the possibility of using zearalenone as an alternative hormone also to cytokinins in winter wheat callus culture.

15.8 Modifying Plant Growth and Yield Using Zearalenone

Our studies show that zearalenone can be used to increase the yield of wheat (Biesaga-Kościelniak et al. 2006a, b). Plants that were sprayed with zearalenone during the heading stage increased their number of grains per ear and their weight per 1,000 grains. Watering and soaking wheat grains produced even better effects in comparison to spraying (Biesaga-Kościelniak et al. 2006a). Zearalenone-treated plants had a higher number and weight per ear and weight per 1,000 grains. The reproduction of plants was also increased. The best results (yield increase) were noted for a zearalenone concentration of 4 mg/dm^3. In soybean cultivation, treating plants with zearalenone also increased their yield (Biesaga-Kościelniak et al. 2006b) (Fig. 15.3). Watering seedlings, soaking seeds and spraying plants increased the yield, the number of pods and the number of grains per pod and per soybean plants. The increase in the yield of soybean and wheat cultivars in comparison to controls (without zearalenone treatment) was 22% and 19% in terms of the number of seeds (grains) and 28% and 24% in terms of the weight of seeds (grains), respectively.

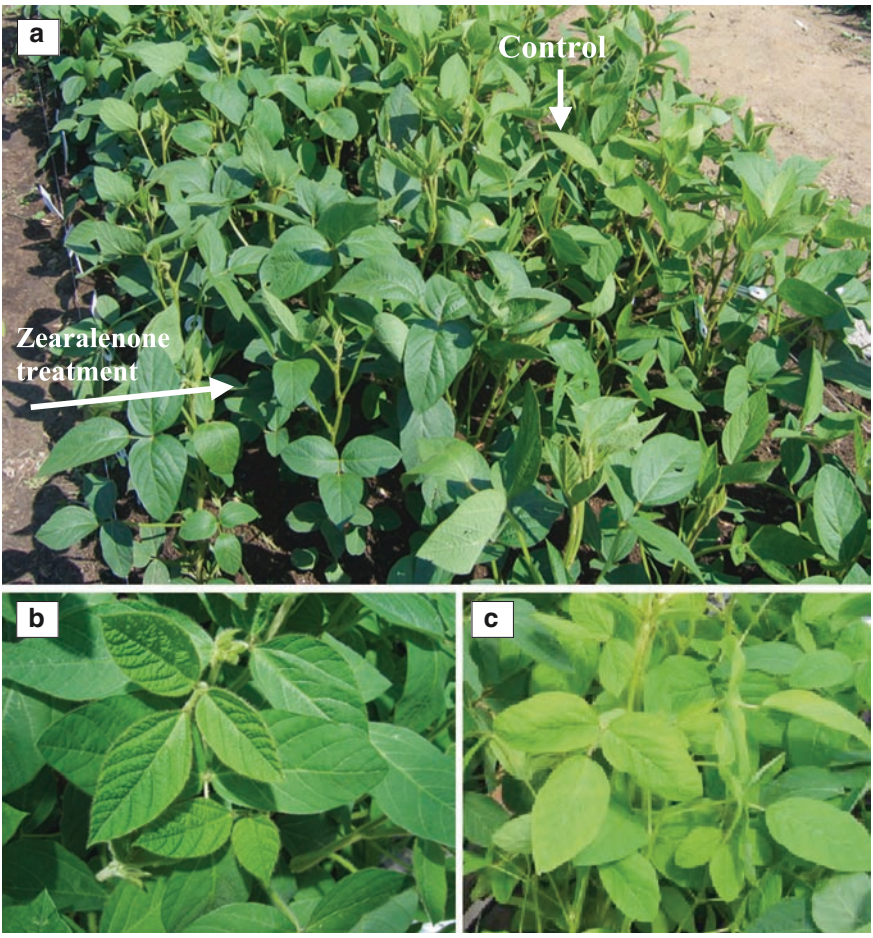

Fig. 15.3 Changes in leaves' shape and height of soybean plants after soaking of seeds in zearalenone solution. (**a**) The picture of the field with plants grown from seeds treated with zearalenone (*left* side) and non-zearalenone treated (control, *right* side). (**b**) Dark-green leaves of soybean plants which were grown from zearalenone-treated seeds. (**c**) Control plants with visible light-green leaves

The effect of zearalenone on crop development may be connected to its influence on the status and functioning of the photosynthetic apparatus (Kościelniak et al. 2008). The after-effects of zearalenone on the growth of soybean and wheat plants, net photosynthesis and transpiration rates, stomatal conductance, photochemical efficiency of photosystem 2 and on final seeds yield were determined. Modifications in leaf area were more pronounced in soybean than in wheat, and this tendency increases in successive developmental phases. The net photosynthesis was stimulated during the juvenile phase and during that of the final one by about 13.6% (average) in soybean plants. Stimulation of transpiration was also observed after

zearalenone treatment on both plant species. The response of CO_2 assimilation in wheat plants was less pronounced when compared to that in soybean. Additionally, the quantum yield of photosystem 2 photochemistry in soybean plants increased rapidly after the seeds were treated with zearalenone, and was higher in wheat plants where this parameter increased constantly during whole period of growth (Kościelniak et al. 2008).

The observed effects of zearalenone action on plant development may be connected to the properties of zearalenone, as a component of mycotoxines. It is known that some stress factors (also toxic chemicals) accelerate plant development and stimulate their generative induction. However, our results (data in preparation) indicate that zearalenone may protect cells from some forms of stress. In drought, stresses induced by either NaCl or changes in water potential (poly(ethylene glycol) content), zearalenone applied in concentrations 2 and 4 mg/dm^3 decreased the inhibiting effect of both these stresses on wheat seedlings and significantly increased the dry mass and length of plants. This effect was especially visible in parts of plants aboveground where an increase of about 84% was detected. In roots, zearalenone stimulated about 42% increase in mass in NaCl conditions in comparison to the control (non-zearalenone-treated) plants. Moreover, at the water potential of −0.5 MPa, the dry mass of shoots in plant cultures treated with zearalenone was 58% higher than that of the control (0 MPa). This protective effect of zearalenone may be a result of its ability to increase the metabolism of seedlings. This effect was confirmed by calorimetric measurements, which indicated an increase in the heat energy emitted by wheat plants treated with zearalenone, where this metabolism parameter increased by about 16% in comparison to non-zearalenone-treated plants.

15.9 Conclusion

Temperature, water availability and light are key climatic factors influencing the production of *Fusarium*. In terms of manipulating environmental conditions to control *Fusarium* spp. diseases, adjustment of soil temperature and moisture has been successfully applied in many countries (Katan 1981; Doohan et al. 2003). Although zearalenone is ubiquitous and toxic, it globally presents a potential danger for animal and human health only when it is absorbed in high amounts or over a long period of exposure (Zinedine et al. 2007). Small amounts of zearalenone act as stimulating factors for plant development, and can serve as plant hormone in induction of physiological processes. Thus, low zearalennone concentration in growth media may be useful to stimulate development of crops and to accelerate the flowering of winter plants, which can be an important factor in agriculture production in changing environmental conditions. A particularly interesting question for future research is the possibility of determining the optimal zearalenone concentration in soil (and in crops) to balance the toxic and favourable action of this substance on both plant development and animal health.

References

Bennett GA, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16:497–516
Bennett GA, Shotweli OL (1979) Zearalenone in cereal grains. J Am Oil Chem Soc 56:812–819
Bernier G, Périllex C (2005) A physiological overview of the genetics of flowering time control. Plant Biotechnol J 3:3–16
Betina V (1993) In: Betina V (ed.) Journal of Chromatography Library, vol 54. Elsevier, Amsterdam, pp 141–252
Biesaga-Kościelniak J (1998) Investigation of the possibility of stimulating generative development of plants by exogenous zearalenone. Acta Physiol Plant 20:4–5
Biesaga-Kościelniak J (2001) Zearalenone as a new hypothetical regulator of plant growth and development. Monograph of Institute of Plant Physiology, Polish Academy of Sciences, Krakow, Poland, pp 1–135
Biesaga-Kościelniak J, Marcińska I, Wędzony M, Kościelniak J (2003) Effect of zearalenone treatment in the production of wheat haploids via maize pollination system. Plant Cell Rep 21:1035–1039
Biesaga-Kościelniak J, Janeczko A, Filek M, Dziurka M, Kościelniak J (2006a) Effect of zearalenone on the growth and productivity of crop plants. I. Effectiveness of application of zearalenone on wheat production. Bibliotheca Fragmenta Agronomica 11:53–54
Biesaga-Kościelniak J, Janeczko A, Filek M, Dziurka M, Kościelniak J (2006b) Effect of zearalenone on the growth and productivity of crop plants. II. Effectiveness of application of zearalenone on soybean production. Bibliotheca Fragmenta Agronomica 11:55–56
Blackwell B, Miller JD, Greenhalgh R (1985) 13C-NMR study of the biosynthesis of toxins by *Fusarium graminearum*. J Biol Chem 260:4243–4247
Burkin AA, Kononenko GP, Sobolewa NA (2002) Group-specific antibodies against zearalenone and its metabolites and synthetic analogs. Appl Biochem Microbiol 38:169–176
Chen XJ, Liu HC, Meng FJ (1989) Direct enzyme-linked immunoassay for zearalenone. Plant Physiol Com 5:61–63 (Chinese, Engl. summ.)
Dai SL, Duan JH, Lu Y, Zhang YH, Cheng JX, Ren J, Zhao XY, Wu YQ, Yu Y, Zuo PP, Wu YY, Ge QS (2004) Phytoestrogen alpha-zearalanol inhibits atherogenesis and improves lipid profile in ovariectomized cholesterol-fed rabbits. Endocrine 25:121–129
Diekman MA, Green ML (1992) Mycotoxins and reproduction in domestic livestock. J Anim Sci 70:1615–1627
Doohan FM, Parry DW, Jenkinson P, Nicholson P (1998) The use of species-specific PCR-based assays to analyse *Fusarium* ear blight of wheat. Plant Pathol 47:197–205
Doohan FM, Brennan J, Cooke BM (2003) Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur J Plant Pathol 109:755–768
Eppley RM, Stoloff L, Trucksess MW, Chung CW (1974) Survey of corn for fusarium toxins. J Assoc Anal Chem 57:632–635
Farnworth ER, Neish GA (1980) Analysis of corn seeds for fungi and mycotoxins. Can J Plant Sci 60:727–731
Frisvad JC, Thrane U (1993) Liquid column chromatography of mycotoxins. J Chromatogr Lib 54:253–372
Fu YF, Meng FJ (1994) Zearalenone in growth and development of winter wheat. Acta Agronomica Sinica 20:271–276 (Chinese, Engl. summ.)
Fu YF, Li HY, Meng FJ (1995) The possible role of zearalenone in the floral gradient in *Nicotiana tabacum* L. J Plant Physiol 147:197–202
Fu YF, Han YZ, Zhao D-G, Meng F-J (2000) Zearalenone and flower bud formation in thin-cell layers of *Nicotiana tabacum* L. Plant Growth Regul 30:271–274
Hagler WM Jr, Towers NR, Mirocha CJ, Eppley RM, Bryden WL (2001) Zearalenone: mycotoxin or mycoestrogen? In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW (eds) Fusarium. Paul E. Nelson memorial symposium. APS Press, St. Paul, MN, pp 21–34

Han YZ, Meng FJ (1986) Studies on zearalenone-like substance of rape plant (*Brassica campestris* L.) and its relation to vernalization. Acta Agricul Univ Pek (Chinese, Engl. summ.) 12:386–388

Han YZ, Meng FJ (1991) Studies on zearalenone influencing the growth and development of *Lemna perpusilla*. Sci Bull (Chinese, Engl. summ) 36:1037–1040

Hans P, van Egmont REC, Marco MAJ (2007) Regulations relating to mycotoxins in food. Perspectives in a global and European context. Anal Bioanal Chem 389:147–157

Hidy PH, Baldwin RS, Greasham RL, Heith CL, McMullen JR (1977) Zearalenone and some derivatives: production and biological activities. Adv Appl Microbiol 22:59–82

Hodge EB, Hidy PH, Wehrmeisters HL (1966) Estrogenic compounds and animal growth promoters. U.S. Patent 3239351. March 8. 3p.

Javier LU, Marazuela MD, Moreno-Bondi MC (2007) Molecularly imprinted polymers applied to the clean-up of zearalenone and α-zearalenol from cereal and swine feed sample extracts. Anal Bioanal Chem 385:1155–1161

Jemmali M, Ueno Y, Ishii K, Frayssinet C, Etienne M (1978) Natural occurrence of trichothecenes, nivalenol, deoxynivalenol, T-2 toxin and zearalenone in corn. Experientia 34:1333–1334

Josephs RD, Schuhmacher R, Krska R (2001) International interlaboratory study for the determination of the *Fusarium mycotoxins* zearalenone and deoxynivalenol in agricultural commodities. Food Addit Contam 18:417–430

Josephs RD, Krska R, MacDonald S, Wilson P, Pettersson H (2003) Preparation of a calibrant as certified reference material for determination of the *fusarium* mycotoxin zearalenone. J AOAC Int 86:50–60

Katan J (1981) Solar heating (solarisation) of soil for control of soilborne pests. Annu Rev Phytopathol 19:211–236

King DT, Kennedy BJ, Pathre SV, Mirocha CJ (1978) Binding characteristics of zearalenone analogs to estrogen receptors. Cancer Res 38:3611–3615

Kościelniak J, Biesaga-Kościelniak J, Janeczko A, Filek W (2009) Can the *Giberella zeae* toxin zearalenone affect the photosynthetic productivity and increase yield formation in spring wheat and soybean plants? Photosyntetica (in press)

Krska R (1998) Performance of modern sample preparation technique in the analysis of *Fusarium mycotoxins* in cereals. J Chromatogr A 815:49–57

Krska R, Josephs R (2001) The state-of-the-art in the analysis of estrogenic mycotoxins in cereals. J Anal Chem 369:469–476

Kuiper GGJM, Lemmen JG, Carlsson B, Corton CJ, Safe SH, van der Saag PT, van der Burg B, Gustafsson J-Å (1988) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor ß. Endocrinol 139:4252–4263

Kuiper-Goodman T, Scott PM, Watanabe H (1987) Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharmacol 7:253–306

Lawrence JF, Scott PM (2000) HPLC methods for the determination of mycotoxins and phycotoxins. Tech Instrum Anal Chem 21:413–456

Li HX, Meng FJ (1989) Isolation and identification of zearalenone from *Apium gaveoleus*. Acta Bot Sin 12:211–215

Li HX, Zhu TX, Zhang C, Li BR, Deng ZP, Li YS, Meng FJ (1980) Studies on zearalenone. Acta Agricul Univ Pek 1:13–28 (Chinese, Engl. summ.)

Magg T, Melchinger AE, Klein D, Bohn M (2002) Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. in grains of transgenic *Bt* maize hybrids, their isogenic counterparts, and commercial varieties. Plant Breed 121:146–154

Maheshwari N, Rajyalakshmi K, Baweja K, Dhir SK, Chowdhry CN, Maheshwari SC (1995) *In vitro* culture of wheat and genetic transformation – retrospect and prospect. Plant Sci 14:49–178

Malekinejad H, Schoevers EJ, Daemen IJ, Zijlstra C, Colenbrander B, Fink-Gremmels F, Roelen BAJ (2007) Exposure of oocytes to the *Fusarium* toxins zearalenone and deoxynivalenol causes aneuploidy and abnormal embryo development in pigs. Biol Reprod 77:840–847

Maragos CM, Appell MD (2007) Capillary electrophoresis of the mycotoxin zearalenone using cyclodextrin-enhanced fluorescence. J Chromatogr A 1143:252–257

Mateo JJ, Mateo R, Hinojo MJ, Llorens A, Jimenez M (2002) Liquid chromatographic determination of toxigenic secondary metabolites produced by *Fusarium* strains. J Chromatogr A 955:245–256

Meng FJ, Que YM, Zhang S-Q (1986) Zearalenone-like substance in winter wheat plants and its relation to vernalization. Acta Bot Sin 28:626–627 (Chinese, Engl. summ.)

Meng FJ, Que YM, Han YZ, Li HX, Wang ZC (1989) Isolation of zearalenone from shoot apices of overwintering winter wheat. Sci China 32:1009–1105 (Chinese, Engl. summ.)

Meng FJ, Han YZ, Que YM, Wang H (1992) Zearalenone, a key substance controlling plant development. Advances in plant regulation. Kluwer, Dordrecht, pp 291–297

Meng FJ, Han YZ, Fu YF, Guo FL (1996) Zearalenone in higher plants. Flowering Newslett 22:54–57

Mesterhazy A, Bartok T, Mirocha CG, Komoroczy R (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Michales SD, Amasimo RM (2000) Memories of winter: vernalization and the competence to flower. Plant Cell Environ 23:1145–1153

Miedaner T (1997) Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed 116:201–220

Miedaner T, Reinbrecht C, Lauber U, Schollenberger M, Geiger HH (2001) Effects of genotype and genotype environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale and wheat. Plant Breed 120:97–105

Miksicek RJ (1994) Interactions of naturally occurring non steroidal estrogens with expressed recombinant human estrogen receptor. J Steroid Biochem Mol Biol 49:153–160

Mirocha CJ, Harrison J, Nichols AA, McClintock M (1968) Detection of fungal estrogen (F-2) in hay associated with infertility in dairy cattle. Appl Microbiol 16:797–798

Mirocha CJ, Christensen CM, Nelson GH (1971) F-2 zearalenone estrogenic mycotoxin from *Fusarium*. In: Kadis S, Alex C, Samuel J (eds) Microbial toxins for fungi and mycotoxins. Academic, New York, pp 727–731

Mirocha CJ, Schauerhamer B, Pathre SV (1974) Isolation, detection, and quantification of zearalenone in maize and barley. J Assoc Anal Chem 57:1104–1110

Muller R, Baier M, Kaiser WM (1991) Differential stimulation of PEP-carboxylation in guard cells and mesophyll cells by ammonium or fusicoccin. J Exp Bot 42:215–220

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nsahlai IV, Green H, Bradford M, Bonsi MLK (2002) The influence of source and level of protein, and implantation with zeranol on sheep growth. Livestock Prod Sci 74:103–112

Parry DW, Jenkinson P, McLeod L (1995) *Fusarium* ear blight (scab) in small-grain cereals – a review. Plant Pathol 44:207–238

Que YM, Liang ZX, Han YZ, Meng FJ (1990) Analysis of zearalenone on winter wheat and cotton plant during flowering and fluiting. Acta Agricul Univ Pek 16:153–155 (Chinese, Engl. summ.)

Richardson KE, Hagler WM Jr, Haney CA, Hamilton RB (1985) Zearalenone and trichothecene production in soybeans by toxigenic *Fusarium*. J Food Prod 48:240–243

Schollenberger M, Muller HM, Rüfle M, Suchy S, Planck S, Drochner W (2006) Natural occurrence of 16 fusarium toxins in grains and feedstuffs of plant origin from Germany. Mycopathologia 161:43–52

Schuhmacher R, Krska R, Grasserbauer M, Edinger W, Lew H (1998) Immuno-affinity columns versus conventional clean-up: a method-comparison study for the determination of zearalenone in corn. Fresenius' J Anal Chem 360:241–245

Scott PM (1993) Recent developments in methods of analysis for mycotoxins in foodstuffs. Trac-Trends Anal Chem 12:373

Shirai Y, Ono Y, Akimoto K (2000) Simultaneous determination of deoxynivalenol and nivalenol in grain by high performance liquid chromatography with multifunctional clean up column for purification. Res Rep Animal Feed 26:1–9

Steyn PS (1995) Mycotoxins, general view, chemistry and structure. Toxic Lett 82/83:843–851

Steyn PS, Thiel PG, Trinder DW (1991) Detection and quantification of mycotoxins in animal feeds by chemical analysis. In: Smith JE, Henderson RS (eds) Mycotoxins and animal foods. CRC Press, Boca Raton, FL, pp 165–222

Stob M, Baldwin RS, Tuite J, Andrews FN, Gillette KG (1962) Isolation of an anabolic uterotrophic compound from corn infected with *Gibberella zeae*. Nature 196:1318–1320

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195–209

Szechyńska-Hebda M, Skrzypek E, Dębrowska G, Biesaga-Kościelniak J, Filek M, Wędzony M (2007) The role of oxidative stress induced by growth regulators in the regeneration process of wheat. Acta Physiol Plant 29:327–337

Tanaka T, Hasegawa A, Yamamoto S, Lee US, Sugiura Y, Ueno Y (1988) Worldwide contamination of cereals by the *Fusarium mycotoxins*, nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. J Agric Food Chem 36:979–983

Tanaka T, Yoneda A, Inoue S, Sugiura Y, Ueno Y (2000) Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry. J Chromatogr A 882:23–28

Urry WH, Wehrmeister HL, Hodge EB, Hidy PH (1966) The structure of zearalenone. Tetrahedron Lett 27:3109–3114

Velluti A, Marin S, Bettucci L, Ramos AJ, Sanchis V (2000) The effect of fungal competition on colonization of maize grain by *Fusarium moniliforme, F. proliferatum* and *F. graminearum* and on fumonisin B and zearalenone formation. Int J Food Microbiol 59:59–66

Vianello A, Macri F (1981) Effect of zearalenone (F-2) on pea stem, maize root, and rat liver mitochondria. Planta 153:443–446

Visconti A, Pascale M (1998) Determination of zearalenone in corn by means of immunoaffinity clean-up and high-performance liquid chromatography with fluorescence detection. J Chromatogr A 815:133–140

Zinedine A, Soriano JM, Molto JC, Jordi Manes J (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol 45:1–18