Introduction

Plant sterols have been reported to include over 250 different sterols in various plants (Moreau et al. 2002). They have been extensively studied in the past years with a major focus on biosynthetic and biochemical aspects (Schaller 2003, 2004, Benveniste 2004). Phytosterol origins are 28- to 32-carbon compounds containing a 24-alkyl group in the side chain derived by transmethylation from *S*-adenosyl-*L*-methionine (Nes 2003). The most common representatives are sitosterol, stigmasterol and campesterol. Phytosterols are primary components of cellular membranes where they regulate fluidity and water permeability. Plant cell membranes incorporate a complicated mixture of sterols. According to Schaller (2004) plant tissues contain an average quantity of 1 – 3 mg of sterols per gram dry weight. ß-Sitosterol is the principle sterol, accounting for 50 % to 80 % of the total sterol content of many plants. For plant species the composition of the sterol mixture is genetically determined; particularly, the ratio of 24-methyl sterols (campesterol) to 24-ethyl sterols (ß-sitosterol) is important (Shaller 2003). Individual plant sterols differ in their effect on membrane stability. Sterols also play a role in cellular differentiation and proliferation. Changes in sterol composition during plant senescence are associated with loss of membrane function (Moreau et al. 2002). Sterol conjugation, the conversion of free sterols to steryl esters, steryl glycosides (sitoindosides) or acetyl steryl glycosides is another potentially important aspect of membrane lipid metabolism (Dyas and Goad 1993).

Compared with other cell membrane systems, the plasma membrane contains the greatest sterol content. A modification in the plasma membrane sterol content affects the properties or modulates the functions of membrane bound proteins such as enzymes, channels, receptors or components of signal transduction pathways, as for instance ATPases. Free sterols are tightly bound to the plasma membrane H^+-ATPase and may be essential for activity of this enzyme. Hormone signaling is affected by variation of the sterol profile mostly because the membrane environment of receptors, channels, etc. had been modified (Lindsey et al. 2003). Sterol interconversions are controlled by phytohormone levels and environmental conditions (light, temperature, water, response to ozone stress, ions etc.), it has been postulated that they are involved in the regulation of membrane properties in response to changing growth conditions (Moreau et al. 2002).

This paper is focused on the effect of plant nitrogen nutrition on free plant sterol content in maize and the changes of its content in relation to sampling period. According to our hypothesis free sterol content in maize can be also affected by period and system of nitrogen application.

Material and Methods

Plant material and cultivation conditions

The effect of nitrogen nutrition for free sterol content in plant was investigated in pot experiment. For experiment, maize seeds (10 seeds of hybrid Rivaldo) were sown into plastic pods containing soil mixture as specified below. The plants (10 plants per pot) were cultivated under natural light and temperature conditions at the experimental hall of the Czech University of Life Sciences Prague, Czech Republic. The water regime was controlled and the soil moisture was kept at 60% MWHC.

For cultivation of maize plants (*Zea mays* L.), 10 kg of Chernozem soil (pH_{KCl} = 7.2, C_{ox} = 1.83 %, CEC = 258 mval kg^{-1}) was thoroughly mixed with N dose applied in the form of ammonium nitrate (AN) for control treatments or was left without nitrogen nutrition for treatments with injected nitrogen application. Nitrogen in the liquid form of urea ammonium

nitrate solution (UAN) was applied into top soil (100 mm depth) on two points of pot 30 days after maize sowing (application by CULTAN system). Each treatment was performed in five replications (Table 1).

Table 1 Design of experiment

Treatment	N rate (g per pot)	Application time
ammonium nitrate 1 (AN1)	2	before sowing
urea ammonium nitrate solution 1 (UAN1)	2	30 days after maize sowing
ammonium nitrate 2 (AN2)	4	before sowing
urea ammonium nitrate solution 2 (UAN2)	4	30 days after maize sowing

The aboveground biomass of maize was sampled after 3, 8 and 13 days after nitrogen application (1, 2 and 3 sampling period).

Analysis of β-sitosterol

Analyses of β-sitosterol from petroleum ether extracts were performed on a HPLC instrument (Waters: Delta 600E multisolvent delivery system, Waters 3996 PDA detector, and Empower 1 PDA software) under the following chromatographic conditions: HPLC column packed with the Ascentis C8 reverse phase (Supelco, 250 mm x 4.6 mm 5μm particle size), using a mixture of the mobile phase A (water) and the mobile phase B (MeOH) at a flow rate of 0.6 $mL.min^{-1}$. UV detection was monitored at 210, 205, 215, 245 and 220 nm. A gradient program (initial conditions of 20% A and 80% B, linearly increasing to 100% B over 15min, holding for 55 min, returning to the initial conditions of 20% A and 80% B over 2 min, and holding for 18 min) was employed.

Results and Discussion

The lower nitrogen content in soil of UAN treatments in the first period of experiment and in following nitrogen application (30 days after maize sowing) affected yield and also total nitrogen content of aboveground biomass (Figures 1 and 2). After application of urea ammonium nitrate (UAN) solution urea is converted into $N\text{-}NH_4^+$ by urease in the soil. The higher concentration of NH_4^+ ion in plant after UAN application (20 % increase of $N\text{-}NH_4^+$ concentration in UAN plants compared to AN plants) could affect plant metabolism as a stress factor. NH_4^+ ion affects membrane activities, strongly decreases membrane potential. *Cruz* et al. (1993) showed that ammonium inhibited the growth of 55 % of a wide range of species in relation to nitrate.

Figure 1 The yield of dry biomass (g per pot)

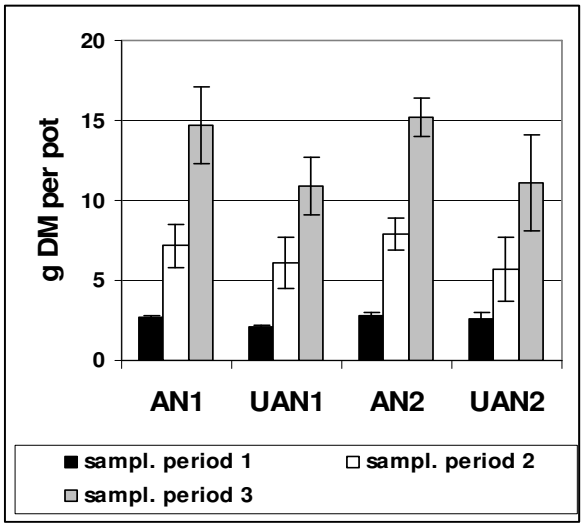
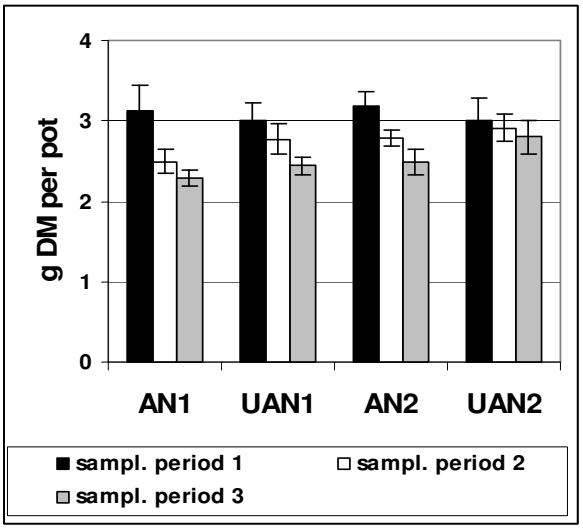


Figure 2 The nitrogen content in maize above ground biomass (mg.kg^{-1})



ß-Sitosterol has been shown to a membrane reinforcer, which regulates acyl chain ordering and water permeability of the phospholipidic bilayers (Ness 2003, Schaller 2004, Banaś et al. 2005). A detailed study of sterol biosynthesis in *Zea mays* has demonstrated that composition of steroids varies as a function of both plant organs and period of development. The changes of ß-sitosterol concentration in maize plants growing in pot experiment were affected by changing growth conditions after UAN fertilizer application. The significant decrease of ß-sitosterol concentration (by 48 % in contrast to 1st period) was determined on UAN2 treatment in the 2nd period (8 days after nitrogen application). Lindsey et al. (2003) confirmed that sterol interconversions are controlled by phytohormone levels and environmental conditions (light, temperature, water, response to ozone stress, ions etc.) and they are involved in the regulation of membrane properties in response to changing growth conditions.

Table 2 ß-Sitosterol concentration in maize plants (µg.g^{-1} DM)

	ß-sitosterol concentration in maize plants (µg.g^{-1} DM)		
	Sampling period		
Treatment	1	2	3
AN1	395.69±68.30	401.61±32.58	434.20±14.11
UAN1	413.22±54.10	410.57±69.87	582.41±58.36
AN2	421.90±75.32	409.00±47.12	379.98±32.47
UAN2	421.07±71.54	201.77±18.21	378.77±41.25

Acknowledgment
A financial support through the grants NAZV 1G58027 and 2B06024 (SUPRAFYT) is gratefully acknowledged.

References

Banaś A, Carlsson AS, Huang B, et al. Cellular sterol ester synthesis in plants is performed by an enzyme (phospholipids:sterol acyltransferase) different from the yeast and mammalian acyl-CoA:sterol acyltransferases. J. Biol. Chem. 2005;280: 34626-34634.

Benveniste P. Biosynthesis and accumulation of sterols. Annu Rev Plant Biol. 2004;55:429-457.

Cruz C, Lips SH, Martin-Louçäo MA. Interactions between nitrate and ammonium during uptake by carob seedlings and the effect and the form of earlier nitrogen nutrition. Physiol. Plant. 1993;89:544-551.

Dyas L, Goad LJ. Steryl fatty acid esters in plant. Phytochemistry. 1993;34:17-29.

Lindsey K, Pullen ML, Topping JF, Importance of plant sterols in pattern formation and hormone signalling. Trends Plant Sci. 2003;8:521-525.

Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in food: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res. 2002;41:457-500.

Ness WD. Enzyme mechanisms for sterol *C*-methylations. Phytochemistry. 2003;64:75-95.

Schaller H. The role of sterols in plant growth and development. Prog Lipid Res. 2003;42:163-175.

Schaller H. New aspects of sterol biosynthesis in grown and development of higher plants. Plant Physiol Biochem 2004;42:465-476.