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PLANT SCIENCES

EFFECT OF SALICYLIC ACID ON ENZYME ACTIVITY IN WHEAT IN IMMEDIATE EARLY TIME AFTER INFECTION WITH *MYCOSPHAERELLA GRAMINICOLA*[*]

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The effect of salicylic acid (SA) on antioxidant enzymes activities in wheat infected with *Mycosphaerella graminicola* was investigated. Different concentrations of SA (0 and 2mM) were sprayed on susceptible and tolerant cultivars of wheat at a two-leaf stage. Enhanced activities of peroxidase, catalase, phenylalanine ammonia lyase, and polyphenoloxidase were determined in two wheat SA-treated cultivars in the presence or absence of pathogen. The results showed that the application of SA was more effective on antioxidant activities than pathogen. However, the highest activities of all tested enzymes were detected in cultivars treated both with SA and pathogen. Although in the earliest time of infection the antioxidant enzymes activities in susceptible cultivar were weaker than in the tolerant cultivar, the enzymes activity enhancement by SA in susceptible cultivar was observable, too. These results suggest SA as plant defense inducer could be an effective agent against *M. graminicola* in wheat.

antioxidant enzymes, salicylic acid, *Triticum aestivum* L.

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INTRODUCTION

Salicylic acid (SA) is a phenolic derivative distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. SA results from decarboxylation of *trans*-cinnamic acid to benzoic acid and its subsequent 2-hydroxylation (Lee et al., 2000). SA belongs to endogenous compounds of phenol nature with plant hormone characteristics (Alvarez, 2003). SA has direct involvement in plant thermogenesis, growth, flower induction, and exchange of ions (O'Donnell el al., 2001). It affects ethylene biosynthesis, stomatal movement, and also reverses the effects of ABA (Abscisic Acid) on leaf abscission (Van Wees, Glazebrook, 2003). In small quantities SA is present in tissues of all plants. Most often the SA present in plant organism is in the form of conjugates, rarely in free. SA induces the development of systemic acquired resistance (SAR) by reactive oxygen species (ROS) production (Kawano, Muto, 2000). Subsequently, SA changes catalase (CAT) and peroxidase (POX) activities (increases or reduces depending on H_2O_2 concentration) (Guan, Scandalios, 2006). CAT and POX are known as a defensive team, targeted at protecting cells from oxidative damage (Mittler, 2002).

POX also has a role in the biosynthesis of lignin and defense against biotic stresses by consuming H_2O_2 in the cytosol, vacuole, and cell wall as well as in extracellular space (Karuppanapandian et al., 2011). The phenolic compounds produced in plants due to pathogen attack are often converted into

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more reactive species by POX and polyphenol oxidase (PPO) (Gómez-Vásquez et al., 2004). They are involved in the oxidation of phenolic compounds in cell wall, suberization and lignifications of host plant cells during the defense reaction against pathogen agents (Welinder, 1992). PPO catalyzing the oxygen-dependent oxidation of phenols to quinines is ubiquitous among angiosperms and is assumed to be active in plant defense against pests and pathogens (Li, Steffens, 2002).

Phenylalanine ammonia lyase (PAL) is involved in the biosynthesis of SA and plays an important role in plant defense (Nugroho et al., 2002; Chaman et al., 2003). In addition, PAL is the primary enzyme in the phenylpropanoid pathway and is the key enzyme in the synthesis of several defense-related secondary compounds such as phenols and lignin (Ngadze et al., 2012).

Zymoseptoria tritici blotch is currently one of the most important foliar diseases of wheat (*Triticum aestivum* L.) worldwide (Bearchell et al., 2005). The causal pathogen *Mycosphaerella graminicola* (Fuckel) J. Schroeter in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz) attacks wheat leaves, causing necrotic blotches which can result in significant yield losses (Forrer, Zadoks, 1983). Recently, fungicides have been largely applied to reduce this disease and associated yield losses; however the recent emergence of resistance/reduced sensitivity among *M. graminicola* populations to the fungicides (Leroux et al., 2007) highlights the urgent need for improved integrated control strategies. Such practices should incorporate cultural control measures, host resistance, chemical application, and biological control.

Antioxidant enzymes play an important role in defense responses of plants to pathogen (Lebeda et al., 2001). The enzymes patterns are intensively studied for cultivar identification in breeding, seed marketing, and in the other fields of agriculture (Samec et al., 1998).

In the present study, the effects of SA and *M. graminicolla* on antioxidant enzymes (CAT, POX, PAL, and PPO) were investigated separately and in combination. The obtained results provide convincing evidence for finding out the possibilities of enhancing the defense mechanism of tolerant and susceptible cultivars of wheat plants in immediate early after pathogen incubation.

MATERIAL AND METHODS

Plant material

In the present study two cultivars of bread wheat (*Triticum aestivum*) were used, one tolerant (Zagros) and the other susceptible (Atrak), based on their pathogenicity testing against *M. graminicolla* by Eslahi et al. (2013).

Preparation of plant extract

Wheat seeds (*Triticum aestivum* L. cvs Zagros and Atrak) were surface-disinfected with 70% alcohol for 5 s and washed twice with sterile distilled water. Then the seeds were sown in 10 cm pots containing a sterilized mixture of field perlite, soil, and leaf compost at a rate of 1 : 1 : 1. The seedlings were grown in a greenhouse under natural condition at 22/18°C (day/night). The pots were irrigated every two days. The pathogen isolate (S1) was received from the Department of Plant Pathology of the Tarbiat Modares University, Iran. S1 was grown on yeast malt extract agar (YMDA) for 5–7 days; spore concentration obtained from the media was adjusted to 10^7 ml^{-1} and supplemented with 0.5% Tween 20. The 12-day-old seedlings were sprayed with various concentrations of SA (0.1, 0.2, 0.5, and 1mM). Each pot was sprayed with 50 ml of the solutions (He, Wolyn, 2005). After 24 h of SA incubation, the plants were inoculated with a suspension of spores of *M. graminicola*. The control treatment was sprayed with sterile distilled water.

Enzyme activities

For measuring enzyme activities of CAT, POX, PAL, and PPO, treated wheat seedlings were used for protein extraction. The plant samples were collected at 0, 3, 6, 12, and 24 h after inoculation. The samples were homogenized in sodium phosphate buffer (0.1 mol l^{-1}, pH 6) and centrifuged at 12 000 *g* for 20 min at 4°C. The supernatants of each fraction were used for the enzymatic activity assay (Sahebani, Hadavi, 2008). For PAL enzyme assay, leaf samples were homogenized in an ice-cooled solution containing 50mM Tris-HCl buffer (pH 8.8) and 15mM β-mercaptoethanol (SIGMA, Germany). The homogenate was centrifuged at 10 000 *g* for 10 min, and the supernatant was collected for enzyme assay. Protein concentration was determined according to Bradford method (Bradford, 1976) using bovine serum albumin as standard protein.

CAT enzyme assay. The activity of CAT was quantified based on the rate of disappearance of the substrate H_2O_2 from the reaction medium containing the protein extract. The H_2O_2 concentration in the reaction medium was quantified by the change in absorbance at 240 nm. The reaction mixture (1 ml) contained 3% H_2O_2 and 50mM phosphate buffer (pH 7), and 0.035 ml was used to measure the decrease in absorbance at 240 nm (Rahnama, Ebrahimzadeh, 2006). Enzyme activity was calculated based on the changes in absorbance at 240 nm (ΔOD) per min per mg of total protein.

POX enzyme assay: POX activity was determined by measuring the appearance of pink/brown colour resulting from guaiacol oxidation in the presence of hydrogen peroxide according to the modified method

Table 1. Effect of treatment with SA and *M. graminicolla* infection agent on the activity of catalase (CAT) in tolerant and susceptible wheat

Treatments	Time after pathogen incubation				
	0 h	3 h	6 h	12 h	24 h
Tolerant wheat					
F	3.2^{bc}	3.9^{b}	4.9^{ab}	4.8^{ab}	5.1^{a}
F+SA	5.7^{c}	7.2^{b}	7.8^{b}	8^{b}	11.3^{a}
N	3.5^{a}	3.8^{a}	3.6^{a}	3.8^{a}	3.54^{a}
N+SA	5.6^{c}	6.35^{bc}	7.1^{b}	7.8^{ab}	8.3^{a}
Susceptible wheat					
F	2.9^{b}	3.1^{b}	3.3^{ab}	3.6^{ab}	3.90^{a}
F+SA	3.52^{c}	4.34^{b}	4.94^{ab}	5.14^{ab}	6.02^{a}
N	3.01^{a}	3.3^{a}	3.2^{a}	3.25^{a}	3.28^{a}
N+SA	3.64^{d}	4.16^{c}	5.06^{b}	5.43^{ab}	5.97^{a}

F = *M. graminicolla*, SA = 2mM salicylic acid, N = Control values are the mean of three replications of changes in absorbance (ΔOD) per min per mg of total protein

$^{a–d}$values in the same row followed by the same letter are not statistically different in Duncan's Multiple Range Test ($P < 0.05$)

of Bania, Mahanta (2012). The reaction mixture contained 5mM guaiacol, 2 ml of phosphate buffer (pH 7.0), 0.2 ml of leaves extract and was initiated with 3% H_2O_2. The changes in absorbance were read at 470 nm in 10-s intervals up to 2 min. Enzyme activity was calculated based on the changes in absorbance at 470 nm (ΔOD) per min per mg of total protein.

Phenylalanine ammonia lyase (PAL) enzyme assay: PAL activity was determined based on the rate of cinnamic acid production as described by Wang et al. (2006). Briefly, 1 ml of the extraction buffer, 0.5 ml of 10mM L-phenylalanine, 0.4 ml of double distilled water, and 0.1 ml of enzyme extract were incubated at 37°C for 1 h. The reaction was terminated by adding 0.5 ml of 6M HCl, and the product was extracted with 15 ml ethyl acetate, followed by evaporation to remove the extracting solvent. The solid residue was suspended in 3 ml of 0.05 ml NaOH and the cinnamic acid concentration was quantified with the absorbance measured at 290 nm. One unit of PAL activity equals to 1mM of cinnamic acid produced per min and expressed as U per mg protein.

Polyphenoloxidase (PPO) enzyme assay: PPO activity was determined according to the method of Shi et al. (2001). The reaction mixture (1 ml) containing 40 µg of protein extract and 10mM phosphate buffer (pH 7.0) was aerated for 2 min in a small test tube, then 100 mm of catechol was added as the substrate. The PPO activity was expressed as a change in absorbance of the reaction mixture at 420 (ΔOD) per min per mg of total protein.

Statistical analysis

All assays were carried out in a completely randomized design. Each treatment consisted of three replicates, and each replicate contained 3 plants for all enzyme activities. Statistical significance was assessed at the level $P < 0.05$. When the analysis was statistically significant, Duncan's Multiple Range Test (SSR Test) was used to test mean separations among mean values of each treatment.

RESULTS

The application of SA resulted in enhancing the activity of all tested enzymes in both infected and uninfected wheat cultivars. The enzyme activity in plants treated with fungus, SA, and their combination increased with time.

CAT: The CAT activity increased through timeline in 0.5 to 2mM SA treatments, reaching the maximum at 24 h after inoculation with pathogen and SA in both cultivars. The activity in 2mM SA treatment was higher than that in other treatments in either the presence or absence of *M. graminicola* inoculation and showed a significant difference. The highest enzyme activity of CAT was observed in the wheat inoculated with pathogen and SA at 24 h after infection in the tolerant and susceptible cultivar with values of 11.3 and 7.23 ΔOD per min per mg protein, respectively. SA and *M. graminicola* increased CAT activity during the entire experiment. In the absence of SA the fungal infection could increase the enzyme activity, but not as effectively as SA. Combination of SA and *M. graminicola* resulted in the sharp increase in CAT activity in tolerant cultivar within 12–24 h and displayed a significant difference to other treatments. On the other hand, the increased activity of the enzyme in sensitive cultivar did not change (Table 1).

POX: The POX activity increased in extracts from SA sprayed wheat at 3 h after pathogen infection and continued for the next 24 h after pathogen challenge.

Table 2. Effect of treatment with SA and *M. graminicolla* infection agent on the activity of peroxydase (POX) in tolerant and susceptible wheat

Treatments	Time after pathogen incubation				
	0 h	3 h	6 h	12 h	24 h
Tolerant wheat					
F	0.63^{c}	0.61^{c}	0.77^{b}	0.86^{ab}	0.91^{a}
F+SA	0.72^{d}	0.89^{c}	1.14^{b}	1.33^{ab}	1.42^{a}
N	0.61^{b}	0.58^{c}	0.69^{a}	0.71^{a}	0.7^{a}
N+SA	0.71^{d}	0.83^{c}	0.97^{bc}	1.07^{b}	1.18^{a}
Susceptible wheat					
F	0.63^{c}	0.61^{c}	0.77^{b}	0.86^{ab}	0.91^{a}
F+SA	0.72^{d}	0.89^{c}	1.14^{b}	1.33^{ab}	1.42^{a}
N	0.61^{a}	0.58^{ab}	0.69^{a}	0.71^{a}	0.7^{a}
N+SA	0.71^{c}	0.83^{c}	0.97^{b}	1.07^{ab}	1.18^{a}

F = *M. graminicolla*, SA = 2mM salicylic acid, N = Control values are the mean of three replications of changes in absorbance (ΔOD) per min per mg of total protein

$^{a–d}$values in the same row followed by the same letter are not statistically different in Duncan's Multiple Range Test ($P < 0.05$)

Table 3. Effect of treatment with SA and *M. graminicolla* infection agent on the activity of phenylalanine ammonia lyase (PAL) in tolerant and susceptible wheat

Treatments	Time after pathogen incubation				
	0 h	3 h	6 h	12 h	24 h
Tolerant wheat					
F	3.4^{e}	5.2^{d}	7.37^{c}	9.6^{b}	12.4^{a}
F+SA	6.85^{e}	9.61^{d}	12.28^{c}	15.57^{b}	18.8^{a}
N	3.25^{a}	3.5^{a}	3.52^{a}	3.35^{a}	3.28^{a}
N+SA	7.02^{e}	8.56^{d}	10.81^{c}	13.45^{b}	15.05^{a}
Susceptible wheat					
F	1.69^{c}	1.58^{c}	3.76^{b}	4.19^{ab}	5.15^{a}
F+SA	2.84^{c}	5.28^{b}	6.03^{ab}	6.97^{ab}	7.96^{a}
N	2.98^{a}	3.12^{a}	3.15^{a}	3.06^{a}	3.01^{a}
N+SA	2.97^{d}	3.96^{cd}	5.26^{b}	6.69^{ab}	7.15^{a}

F = *M. graminicolla*, SA = 2mM salicylic acid, N = Control alues are the mean of three replications of changes in absorbance (ΔOD) per min per mg of total protein

$^{a–e}$values in the same row followed by the same letter are not statistically different in Duncan's Multiple Range Test ($P < 0.05$)

The activities were more intensive in tolerant cultivar compared to susceptible cultivar and significantly differed. The maximum POX activity was observed in the inoculated wheat with pathogen and SA at 24 h after infection in the tolerant and susceptible cultivar with the value of 2.74 and 1.78 ΔOD per min per mg protein, respectively (Table 2).

PAL: The enzyme activity of PAL had the same pattern as that of POX. PAL activity increased in the SA sprayed wheat at 3 h after pathogen infection. Activity of PAL in both SA sprayed and infected plants increased significantly compared to control plants. However, in combination treatment the PAL activity was more enhanced than in the other treatments and showed a significant difference. In general, the activity of PAL was increased in the SA treatment and pathogen inoculation and the peak appeared 24 h after pathogen challenge (Table 3).

PPO: The PPO activity increased in the SA sprayed wheat and the increase continued 24 h after pathogen challenge in tolerant more than in susceptible cultivar. Although in pathogen inoculated plants the PPO activity was increased significantly compared to control plants, the increase was lower if compared to activities of infected and SA sprayed wheat. The PPO activity in tolerant cultivar sharply increased

Table 4. Effect of treatment with SA and *M. graminicolla* infection agent on the activity of polyphenol oxidase (PPO) in tolerant and susceptible wheat

Treatments	Time after pathogen incubation				
	0 h	3 h	6 h	12 h	24 h
Tolerant wheat					
F	3.4^{e}	5.2^{d}	7.37^{c}	9.6^{b}	12.4^{a}
F+SA	6.85^{e}	9.61^{d}	12.28^{c}	15.57^{b}	18.8^{a}
N	3.25^{a}	3.5^{a}	3.52^{a}	3.35^{a}	3.28^{a}
N+SA	7.02^{e}	8.56^{d}	10.81^{c}	13.45^{b}	15.05^{a}
Susceptible wheat					
F	0.2^{c}	0.23^{bc}	0.27^{b}	0.32^{ab}	0.4^{a}
F+SA	0.24^{c}	0.42^{b}	0.46^{ab}	0.51^{a}	0.56^{a}
N	0.25^{b}	0.27^{ab}	0.32^{a}	0.33^{a}	0.31^{a}
N+SA	0.26^{c}	0.39^{b}	0.43^{ab}	0.46^{a}	0.48^{a}

F = *M. graminicolla*, SA = 2mM salicylic acid, N = Control values are the mean of three replications of changes in absorbance (ΔOD) per min per mg of total protein

$^{a–e}$values in the same row followed by the same letter are not statistically different in Duncan's Multiple Range Test ($P < 0.05$)

3 h after the SA treatment and this enhancing was observed in both infected and uninfected plants with a significant differerence (Table 4).

DISCUSSION

In this study two wheat cultivars with a different level of tolerance against *M. graminicola* – Atrak (as susceptible) and Zagros (as tolerant) – were used. The aim of this study was considering defense strategies in wheat against *M. graminicola* in the early time after infection with pathogen in the presence and absence of SA.

The increase in enzyme activities was reported in many plant–abiotic and plant–microorganism interactions (Chutia et al., 2012; Cass et al., 2015). It is regarded as a general defense response of the organism to the effect of stress (Dikilitas, 2003).

Phenylpropanoids belong to the largest group of secondary metabolites (including lignin, phytoalexins, tannins etc.) produced by plants in response to biotic and abiotic stresses (Vogt, 2010). During compatible and incompatible interaction between pathogen and plants, the shikimic acid pathway is involved in plant defense due to breakdown products such as lignin, phenolics, and phytoalexins (Somssich, Hahlbrock, 1998; von Forell et al., 2015). PAL and POX play important roles in biosynthesis of phenolics, phytoalexins, and lignin, the three key components responsible for disease resistance. POX decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin and defense against biotic stresses by consuming H_2O_2 in the cytosol, vacuole, and cell wall as well as in extracellular space (Gill, Tuteja, 2010; Karuppanapandian et al., 2011). PAL catalyzes the conversion of phenylalanine to *trans*-cinnamic acid, a key intermediate in the synthesis of salicylic acid (Ramamoorthy et al., 2002).

There are numerous studies that investigate the strategy of over activation of antioxidant enzymes with the ultimate aim of enhancing stress resistance in plants.

Maksimov et al. (2013) showed that the wheat infection with *S. nodorum* strain caused the enhanced transcriptional activity of the CAT gene and the increased synthesis of its product. Our results showed that the maximum activity of CAT (11.3) was observed after 24 h of pathogen infection in Zagros (tolerant cultivar) when sprayed with 2mM of SA. Anahid et al. (2013) showed that the amount of CAT and superoxide dismutase activity increased significantly in tolerant cultivars, and also in susceptible wheat cultivars infected with *Puccinia striiformis* f.sp. *tritici* and this amount in susceptible cultivars was lower than in tolerant cultivars.

The activity of POX and PAL enzymes was enhanced in pepper roots during interactions with *Verticillium dahliae* (Idoia et al., 2006). Koche, Choudhary (2012) showed that the activities of chitinases, 1,3-glucanases, and PAL were higher in tolerant cultivars of mungbean seedlings when challenged with *Cercospora canescens*. This induced activity of defense related enzymes was found to be associated with the tolerance status of mungbean cultivars. In the present research, PAL activity was significantly higher in all treatments of the tolerant cultivar than of the susceptible one. PAL activity showed increasing trend from the first time points (at the time of infection) to 24 h after inoculation, in tolerant and susceptible cultivars.

Nawar, Kuti (2003) showed that there are positive relationships between the POX isozymes and resistance development in plants. Furthermore, Caruso et al. (2001) experimentally supported the

idea that POX plays a defense role against invading pathogens of wheat kernels. They planned to determine changes in POX activity and newly developed POX isozymes in plants pre-sprayed with salicylic, benzoic, citric, and oxalic acids, by using ribavirin as the inducer. The involvement of POXs in plant defense responses against pathogens has repeatedly been reported. For wheat POXs, it was observed that the POXs could bind to chitins and germinating spores of the fungus *T. caries*. Specific chitin-binding isozymes of POX play an important role in pathogenesis of plant diseases caused by fungi (K h a i r u l l i n et al., 2000).

A direct evidence for the role of PPO in inhibiting pathogen ingress or growth comes from challenging transgenic tomato plants with enhanced or suppressed PPO levels, by the bacterial pathogen *Pseudomonas syringae* pv *tomato*. During the infection process, in PPO over-expressing plants a reduction in bacterial growth was detected, whereas PPO anti-sense-suppressed lines supported greater bacterial numbers (T h i p y a p o n g et al., 2004). The pathogen-induced PPO activity continues to be reported for a variety of plant taxa, including monocots and dicots (e.g. C h e n et al., 2000; D e b o r a h et al., 2001). Similarly, studies describing correlations of high PPO levels in cultivars or lines with high pathogen resistance continue to provide support for the pathogen defense role of PPO (R a j et al., 2007). Our results showed that the PPO activity of tolerant cultivar was significantly higher than that of susceptible cultivar in all time points. It was also reported that the activity of PPO and POX increases in roots of wheat when infected with *Ustilago tritici*, in early and later stage of the disease development (A n j u m et al., 2012).

In our study, treatment with SA enhanced the activity of all four enzymes (CAT, POX, PAL, and PPO) during 24 h after the plants infection with *M. graminicola*. The specific SA action on the defense enzymes activity in different plants is due to different isoforms of the given enzyme that are activated by SA in different ways (G u a n , S c a n d a l i o s , 2006). In the present research, SA treatment was more effective in increasing enzymes activity compared with the use of *M. graminicola* during 24 h after inoculation. However, treatment with SA combined with pathogen induced the highest activity of antioxidant enzymes. When citric and benzoic acids were used as inducer against faba bean chocolate spot disease, these components were very effective and resulted in the lowest percentages of disease severity and the highest levels of POX activity (Hassan et al., 2007). This increase reached up to two-folds of the control treatment.

According to our results, the SA treatment increased the activity of all tested enzymes in both the tolerant and susceptible cultivars. From the beginning of the experiments, the enzymes activity was more intensive in the tolerant cultivar than in the susceptible one. SA had greater impact on the increase of enzymes activity than pathogen in the susceptible cultivar. Consequently, even in susceptible cultivars SA can be used as a resistance inducer.

Overall, in this paper, four enzymes activity related to the defense system was performed in the inoculated wheat with pathogen as well as the induction agent defense system SA in the early hours. The main objective was to study the effect of SA on the activity of these enzymes in tolerant and susceptible cultivars in the immediate early hours after incubation with pathogen.

CONCLUSION

The application of SA resulted in enhancement of enzymes activity in both resistant and susceptible plants during examination periods. The role of oxidative enzymes such as CAT, POX, and PPO could be explained as an oxidation process of phenol compounds to oxidized products (quinines) which may limit the fungal growth. Finally, our results showed that induction of resistance with SA affected the defense enzymes activity. Due to the deleterious effects of chemical control on human health and environment, using such alternative substances to manage *M. graminicola* and other pathogens is very promising in the future.

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GERMAN SHEPHERD DOG MILK COMPOSITION AND ITS CHANGES DURING LACTATION*

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Milk composition of nine lactating German Shepherd nursing females was studied. The experiment took place at the breeding facility of Police of the Czech Republic (breeding centre for service dogs in Domažlice) in days 2–30 of the females' lactation. Females were given a commercial granulated feeding mixture (starter category). Canine milk samples were analyzed using an infra-red instrument MilkoScan FT 120. Calculated mean values for colostrum (day 2 *postpartum*) and normal milk (days 4–29 *postpartum*) were: 23.86 and 24.63% for total solids, 8.14 and 7.22% for protein, 6.04 and 5.76% for casein, 10.22 and 11.32% for fat, 3.40 and 4.48% for lactose. German Shepherd milk showed almost no variation in composition after day 4 of lactation. Differences observed between colostrum and normal milk composition were statistically insignificant but not for lactose. No significant differences in density and other composition parameters between colostrum and normal milk were identified.

colostrum, normal milk, fat, protein, lactose, total solids

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INTRODUCTION

Milk provides a complete diet for the new-born mammals. Milk composition varies widely across species with the stage of lactation (Farrell et al., 2006) and is influenced by multiple factors including the breed, health status of the mammal, feeding practices, and the time of milk samples collection *postpartum* (Bernabucci et al., 2013).

Colostrum is the first milk produced after birth and is particularly rich in immunoglobulins, antimicrobial peptides, and other bioactive molecules, including growth factors. It is important for the nutrition, growth, and development of newborns and contributes to the immunologic defense of neonates (Playford et al., 2000). Bitches produce colostrum, which is more abundant in whey proteins (immunoglobulins) than mature milk, during 27–72 h immediately after birth (Segalini, 2008). Colostrum composition depends on the intensity that new-born mammals suck and varies with time after the birth, most significantly within 24 h after parturition (Bernabucci et al., 2013). Heddle, Rowley (1975) reported that the total immunoglobulin concentration in dog (mongrels) colostrum is 15 mg/ml on the day of parturition and 2–3 mg/ml on day 3 after parturition compared to 1–2 mg/ml for normal milk. However, the time of samples collection and rearing conditions were not described in this study. Costăchescu et al. (2011) compared the chemical composition of colostrum of German Shepherds, Caucasian Shepherds, Golden Retrievers, Labrador Retrievers, and West Terriers

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reared under the same conditions and with the same care. The samples of colostrum were collected from 10 females of each breed on day 2 after parturition and had the following average characteristics: fat 8.42%, protein 5.97%; water 75.77%, pH 6.0. German Shepherd colostrum was the richest in protein and the poorest in fat in comparison with other dog breeds. Rüsse (1961) recorded that total protein decreases, whereas fat and lactose contents rise during the bitch colostral period.

After the colostral period, mammalian females produce mature milk consisting of an emulsion of fat and a suspension of casein micelles (caseins – the major milk proteins, calcium, phosphorus), all suspended in an aqueous phase which contains solubilized lactose, whey proteins, and some minerals (Bernabucci et al., 2013). In the past only a few studies focused on dog milk composition and its changes during lactation and widely differing values were reported. They were mostly published before 1990 (and not in English) (Abderhalden, 1899; Mundt et al., 1981; Kienzle et al., 1985). According to Oftedal (1984), dog milk contained 21–26% total solids; 8–12% fat, 7–10% protein on average. Scantlebury et al. (2000) observed that the milk of Labradors (n = 15) and Schnauzers (n = 6) from the Waltham Centre for Pet Nutrition fed by commercial diet contained on average 21.2% total solids, 8.23% crude protein, 8.11% fat, and 4.86% saccharide in days 24–30 of lactation with no difference among breeds. Also Rüsse (1961) recorded no differences in basic milk composition between breeds but Costăchescu et al. (2011) described differences in normal milk composition among five dog breeds. Oftedal (1984) reported the average contents of 22.7% total solids, 9.47% fat, 7.53% protein, and 3.81% saccharide for the milk of five Beagle bitches from a research colony with no changes in milk fat and protein concentration from day 7 to day 37 and with the increase of saccharide content from day 7 to day 30 of lactation. Luick et al. (1960) found out higher values (26% total solids, 9.8% protein, 13% fat) and Lönnerdal et al. (1981) lower values (5.31% protein, 4.66% fat, 4.81% saccharide) for Beagle milk. These differences may be explained by differences in analytical methods, in the number and collection time of samples or in feed composition of the observed bitches.

The studies focused on dog milk composition and its changes during lactation are essential for understanding the nutrition of neonatal dogs and for developing new puppy milk replacement formulas (Lönnerdal et al., 1981). Although the replacers should be formulated to reflect the nutrient composition of dog milk, some of them contain either insufficient or excessive amounts of some nutrients (such as protein, fat, calcium, phosphorus), which can cause health problems in puppies (Adkins et al., 2001).

The German Shepherd is the most frequent breed and the most commonly used dog for police and military purposes in the Czech Republic. But only little data about German Shepherd milk composition during lactation is available. Therefore, the aim of the study was to analyze the composition of dog milk of this breed and describe the changes during lactation.

MATERIAL AND METHODS

Experimental animals

Nine German Shepherd females were used in this study. The experiment took place in the breeding centre for service dogs (breeding facility of Police of the Czech Republic) in Domažlice during the first thirty days of the females' lactation. After this period the puppies were mainly given supplemental food. The females were given a commercial granulated feed mixture Royal Canin (sensible category) (28% protein, 18% fat, 2.8% fibre, 34.2% starch, 5.5% ash). The females were fed 2–3 times a day receiving 600 g of feed daily. The number of puppies in one litter was 6.43 ± 0.98. The puppies were born in the period June 17th–November 13th.

Milk samples

Individual milk samples were taken by manual milking before noon feeding of females from randomly selected nipples of each female. Before milking the nipples were wiped with a warm wet cloth and then massaged. The milking took 2–7 min. Puppies were moved away from bitches during the sampling. Totally 54 samples were taken between 9:00 and 13:00 on the days indicated in Table 1. The first milk samples were taken 45–51 h after the puppies' delivery. Milk or colostrum were collected into standard 35-ml polypropylene milk sample vials with caps and immediately frozen to –18°C and delivered in cooling containers to the university laboratory for the analysis of basic milk parameters (fat, protein, lactose, total solids, casein, milk density). The samples were preheated to 40 C in a water bath before the analysis.

Instrumental analysis

Canine milk samples (each sample was measured twice) were analyzed using the infra-red apparatus MilkoScan FT 120 (Foss Electric, Hillerød, Denmark) calibrated by an external accredited laboratory using ten cow milk standard samples. The calibrated components in these standard samples were determined using reference methods recommended by Foss com-

Table 1. Parameters of German Shepherd milk during the first 30 days of lactation period

Parameter	Days of lactation					
	2	4	7–8	14–15	21–22	27–29
Milk density (g cm^{-3})	1.027 ± 0.004	1.027 ± 0.003	1.032 ± 0.004	1.032 ± 0.005	1.034 ± 0.006	1.035 ± 0.002
Casein (%)	6.04 ± 1.15	4.90 ± 0.51	5.62 ± 0.49	5.83 ± 0.80	6.28 ± 1.04	6.15 ± 0.12
Protein (%)	8.14 ± 1.55	6.37 ± 0.48	7.08 ± 0.56	7.30 ± 1.00	7.83 ± 1.38	7.51 ± 0.25
Fat (%)	10.22 ± 1.49	10.29 ± 1.54	10.75 ± 1.20	11.47 ± 1.72	12.26 ± 3.01	11.84 ± 0.33
Lactose (%)	3.40 ± 0.40^{a}	4.14 ± 0.25^{b}	4.38 ± 0.24^{b}	4.43 ± 0.32^{b}	4.58 ± 0.29^{b}	4.84 ± 0.46^{b}
Total solids (%)	23.86 ± 1.99	22.18 ± 1.94	23.71 ± 1.50	24.85 ± 2.05	26.35 ± 3.30	26.05 ± 0.65

^{a,b}means for lactose with the same subscript do not differ by more than significant level $\alpha = 0.05$; no significant differences concerning other parameters were found

pany (fat: Gerber method, protein: Kjeldahl method, lactose: Megazyme Lactose-Galactose enzymatic test kit, total solids and solids-non-fat: Heating Owen method at 102°C, freezing point: cryoscopic method).

Statistical methods

Means of eight milk samples taken on the same day of lactation were calculated with their standard deviations. The mean for the whole period of lactation except colostrum period was calculated as well. Milk component values were processed by the analysis of variance (ANOVA). The Scheffé's test was used to evaluate statistically significant differences between dog milk composition values during lactation. Statistical analyses were performed using SAS software, Version 9.3.

RESULTS

The results of the German Shepherd milk composition changes during the first 30 days of lactation expressed as the mean of 9 individual samples ± standard deviation are given in Table 1. In our study the mean values for colostrum (day 2 *postpartum*) and normal milk (calculated from day 4 to days 27–29 *postpartum*) were: 23.86 and 24.63% total solids, 8.14 and 7.22% protein, 6.04 and 5.76% casein, 10.22 and 11.32% fat, 3.40 and 4.48% lactose, respectively. The changes of normal milk produced from day 4 *postpartum* were not significant.

However the protein content increased from day 4 till days 21–22 of lactation by 1.46%, and then slightly decreased (till days 27–29). The amount of casein as a proportion of total protein content increased from 64.2% (day 4) to 81.9% (days 27–29) in our experiment.

Similarly to protein, the fat content increased from day 4 till days 21–22 of lactation by 1.97%, and then decreased slightly by 0.32%.

In our study, the lactose content increased throughout the entire observation period with significant differences between colostrum and milk lactose content ($P < 0.05$).

There were no significant differences in density between colostrum and normal milk; the mean value of normal milk density was 1.032 g cm^{-3}.

DISCUSSION

This study is unique owing to two significant aspects: (1) sufficiently large sets of milk samples were collected from the same breed and (2) standardized breeding conditions were provided for the females by the breeding facility of Police of the Czech Republic in Domažlice – a unique institution of its kind in Europe. These conditions enabled us to obtain data on German Shepherd milk composition and its statistical evaluation.

The mean values for colostrum and normal milk noted in our study correspond with the values of Beagle bitches' milk composition reported by Oftedal (1984). Higher protein and carbohydrate (lactose) contents and lower total solids and fat content of colostrum were also noted in other dog breeds when compared with normal milk (Segalini, 2008; Costăchescu et al., 2011), although the variability of results is naturally large.

Unfortunately, there is very little data about dog colostrum composition in scientific literature. Costăchescu et al. (2011) reported very similar values concerning total solids and protein contents (24% and 8.1%), but lower values of fat (6.5%) in German Shepherd colostrum (2 days *postpartum*) if compared to the present experiment. In Beagle colostrum Adkins et al. (2001) noted higher content of protein (14%) and fat (13%) and lower content of lactose (2%) (1 day *postpartum*).

Like in our experiment, Lönnerdal et al. (1981) described an increase in protein content throughout the lactation period of Beagles but with lower values: from 4.3% (days 0–10) to 5.31% (days 21–30). On the other hand, it was recorded that the protein content decreased from 7.5% on day 10 to 5.1% on

day 25 for German Shepherds (Costăchescu et al., 2011).While in our experiment the amount of casein as a proportion of total protein content increased, Adkins et al. (2001) noted a higher percentage of casein (75.4%) in the early days of Beagle lactation and a lower percentage (69.5%) by day 28 (with non-significant differences).

Similarly to our data, the increase of fat content from day 10 till day 25 was reported for German Shepherds (Costăchescu et al., 2011) as well as Beagles (Lönnerdal et al., 1981). Oftedal (1984) and Adkins et al. (2001) observed the opposite tendency in the fat content of Beagle milk but with nonsignificant differences. Oftedal (1984) recorded that the fat content decreased from 10.9% (days 7–9) to 8.7% (days 22–23), and then increased to 9.16% (days 29–30). The author explained that the variation among studies may be the function of a sampling analytical bias or that this may represent the actual difference among dogs. Also some other factors like females' feed composition and energy intake in relation to their requirements as well as yield itself could be the reason for certain discrepancies in the existing literature.

In Beagles, the increasing values of lactose content during the first 30 days of lactation were confirmed by other authors (Oftedal, 1984; Adkins et al., 2001). Luick et al. (1960) noticed no differences in lactose content in Beagle milk measured on days 15 and 30 *postpartum*. However we noticed changes in lactose content during the first days of lactation in German Shepherds.

Like in other animals, differences in the density of colostrum and normal milk were insignificant. Density value is generally decreased by fat and increased by other non-fat solid compounds (proteins, lactose, and minerals). Our results therefore indicate that the effect of the fat content increase (by 0.07%) was compensated by the increase of non-fat solids (by 0.6%).

CONCLUSION

In conclusion, the present analysis of a reasonably large set of German Shepherd milk samples yielded novel unique data. German Shepherd milk showed almost no variation in composition after day 4 of lactation. Statistically significant differences in composition between colostrum and normal milk were observed only in the case of lactose. The obtained results enabled us to verify the published data about dog milk composition and provided new information on German Shepherd milk composition during lactation. Comparing the obtained results and the literature data on the composition of dog milk, it is evident that additional experiments focused on German Shepherd milk are needed. This is especially important in view of developing a puppy formula as a replacement for mother's milk.

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REPELLENCE AND ATTRACTION OF *APIS MELLIFERA* FORAGERS BY NECTAR ALKALOIDS*

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Plant secondary metabolites present naturally in nectar, such as alkaloids, may change the behavioural responses of floral visitors and affect pollination. Some studies have shown that nectar containing low concentrations of these secondary metabolites is preferred by honey bee foragers over pure nectar. However, it remains unclear whether this is caused by dependence or addictive behaviour, a simple taste preference, or by other conditions such as self-medication. In our choice experiment, free-flying bees were presented with artificial flowers holding 20% sucrose containing 0.5–50 μg ml^{-1} of one of the naturally occurring nectar alkaloids – caffeine, nicotine, senecionine, and gelsemine. Nectar uptake was determined by weighing each flower and comparing the weight to that of the control flower. Our experimental design minimized memorizing and marking; despite this, caffeine was significantly preferred at concentrations 0.5–2 μg ml^{-1} over control nectar; this preference was not observed for other alkaloids. All of the compounds tested were repellent at concentrations above 5 μg ml^{-1}. We confirmed previous reports that bees exhibit a preference for caffeine, and hypothesize that this is not due only to addictive behaviour but is at least partially mediated by taste preference. We observed no significant preference for nicotine or any other alkaloid.

nectar preference, caffeine, nicotine, senecionine, gelsemine

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INTRODUCTION

A characteristic feature of higher plants is their capacity to synthesize a variety of organic molecules known as secondary metabolites, which can protect them against a wide variety of pests (Wink, 1988). Several adaptive hypotheses have been proposed to explain the ecological and evolutionary roles of secondary metabolite alkaloids in nectar. They may deter nectar robbers (Johnson et al., 2006), prevent microbial degradation of nectar (Herrera et al., 2009), enhance cross-pollination by encouraging pollinators to move more quickly among flowers (Adler, 2000; Kessler, Baldwin, 2007), permit insect self-medication (Gherman et al., 2014; Baracchi et al., 2015), or even enhance connections between plants and certain insect species by eliciting addictive behaviour (Renwick, 2001). The effect of alkaloids on bee colony fitness and mortality has been tested in several studies (Singaravelan et al., 2005; Gegear et al., 2007; Reinhard et al., 2009; Köhler et al., 2012; Cook et al., 2013; Manson et al., 2013), which suggest that alkaloids provide benefits to weak colonies under certain circumstances. Some studies show that bees prefer nicotine and caffeine in choice experiments, perhaps because they develop dependence to these compounds (Thomson et al., 2015). Despite the possible evolutionary and ecological implications,

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the concurrent effects of floral attractiveness and bee preference on pollinator visitation have not been widely studied. These studies are important because diet has a significant effect on pathogen infections in animals and the consumption of secondary metabolites can either enhance or mitigate the severity of infections (Manson et al., 2010).

The present study investigates the influence of secondary metabolites in floral nectar on nectar preferences in pollinators by measuring the preference of *Apis mellifera carnica* for various concentrations of secondary metabolites that are known to be present in nectar (caffeine, senecionine, nicotine, and gelsemine) in artificial flowers.

MATERIAL AND METHODS

The design of the experiment followed that of Gegear et al. (2007), with several modifications. For the behavioural assay, nectar (20% sucrose solution) containing nicotine, caffeine, gelsemine, and senecionine as a free base (Sigma-Aldrich, St. Louis, USA) was used in artificial flowers.

The artificial flowers were constructed by attaching 2.5 cm wide yellow cardboard rims to the mouths of 1.5 ml microcentrifuge tubes. These flowers were weighed, filled with nectar solutions, and placed in a spiral formation on a 70 × 70 cm green Styrofoam board. Two independent overlapping concentration sets (0–0.5–1–2–5.5 and 0–0.5–2–5.5–17–50 µg ml^{-1}) were tested and later pooled for statistical evaluation. Each compound was used in triplicate per set and each set was tested in five or six independent experimental replicates, resulting in n = 15, 18, or 33 for each data point. The experimental concentration range was thus 0.5–50 µg ml^{-1}. Control flowers contained only a 20% sucrose solution.

Each flower held 1.2 ml of nectar. The green board was placed 1 m from the entrance of an outdoor hive housed in a bee-proof flight enclosure (3 × 4 × 2.5 m). The hive was housed in this enclosure for 1 week prior to the experiment, and the *Apis mellifera carnica* were supplied with pollen and honey frames during this time. No natural sources of nectar or pollen were available to the bees. At the beginning of the experiment, the bees were stimulated by dusting approximately 300 mg of pollen over the green board. The approximate volume of the solution in the control flowers was monitored over the course of the experiment, and the experiment was terminated when this volume dropped below 500 µl (which took approximately 60–90 min). The difference in the weights of the artificial flowers before and after the experiment was used to calculate the volume of nectar that was removed by the bees.

Experimental replicates were conducted twice a day, in the morning and in the afternoon, in July 2013. Between each replicate, flowers were re-filled and their positions were newly randomized. No further data filtering was applied.

Statistical analysis was done using General Linear Models followed by Dunnett's (2-sided) post-hoc multiple comparison test using the IBM SPSS Statistics (Version 20.0, 2012).

RESULTS

In the present study, honey bees preferred caffeine concentrations between 0.5–2 µg ml^{-1}, with by up to 22% higher uptake from the flowers containing 2 µg ml^{-1}of nectar (121.7% ± 7.0% SEM, n = 33, P = 0.045) than from the control flowers. The other alkaloids tested did not show this effect, and the attraction of all lower concentrations of the alkaloids to foraging bees was comparable to that of the control flowers (Fig. 1). In concentrations higher than 5.5 µg ml^{-1}, all compounds were repellent (P < 0.05). Caffeine and nicotine were slightly better tolerated than gelsemine and senecionine, which showed more significant repellence at 17 µg ml^{-1}. In the highest concentration tested (50 µg ml^{-1}), nectar uptake was approximately zero for all compounds.

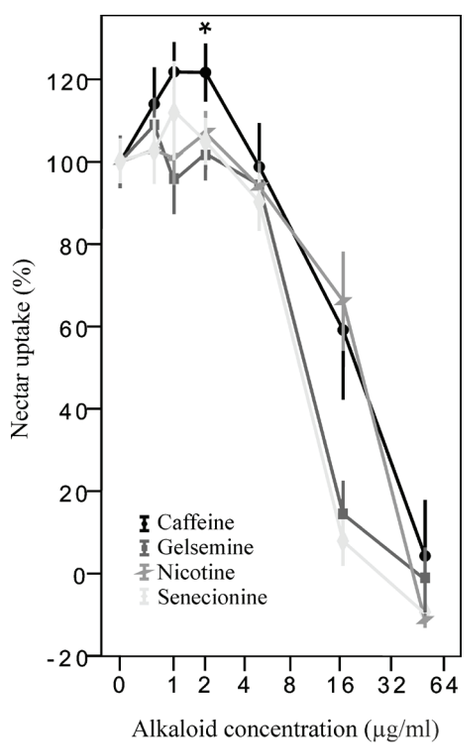

Fig. 1. Average uptake of artificial nectar containing alkaloids based on their concentration. Each point represents means of 15–33 independent replicates
*statistically significant value from the control (P < 0.05)

DISCUSSION

Singaravelan et al. (2005) found that bees preferred 25 ppm of caffeine in artificial nectar compared to sugar solution only, which reflects the amounts naturally present in nectar of citrus flowers (11.61–94.26 ppm). In the same study, the presence of nicotine in nectar (at concentrations of 0.5 and 1 ppm) also elicited a significant feeding preference. Bees have also been shown to prefer nicotine derivatives, such as the neonicotinoids used for pest control (Kessler et al., 2015), which may have negatively affect their health.

We confirmed the preferential behaviour of bees towards caffeine but not towards nicotine. Moreover, neither of the two other alkaloids tested, senecionine and gelsemine, was preferred over the control. This shows that the preference of bees for caffeine (and for nicotine based on previous studies) is relatively specific for these alkaloids. This study differed from previous studies (such as Gegear et al., 2007) in the design and in the randomization of the flowers; we also changed the flower rims after certain experimental sets to prevent the bees from forming associations between floral colour and position and nectar properties. This was done in order to reduce the number of addicted individuals, as addictive behaviour has been previously recognized in insects (Bainton et al., 2000; Schafer, 2004). Under our experimental conditions, preference was expressed not as an increase in the frequency of visits to a flower, but rather as an increase in feed intake per visit. Interestingly, in this experiment, the preference for caffeine was observed at 10-fold lower concentrations than in the study by Singaravelan et al. (2005).

The results of this experiment support the theory that the long-term preference for caffeine and nicotine is based on addiction rather than unintentional self-medication (Gherman et al., 2014; Baracchi et al., 2015). Certain dietary elements appear to suppress the development of taste sensitivity to deterrents in insects, while the presence of specific stimulants in the diet may result in the development of dependence on these compounds (Renwick, 2001). Moreover, this suggests that taste preference depends on the presence of other compounds or concentrations simultaneously offered in nectars during experiments, or in surrounding forage. This is supported by studies in which simultaneous testing of different ranges of concentrations resulted in different preferential responses (Singaravelan et al., 2005).

To the best of our knowledge, this is the first time senecionine (as a free base) has been used in preference studies. According to our results, the presence of senecionine as a hazardous honey pollutant cannot be explained by the preferential behaviour of honey bees towards senecionine-containing flowers. Similarly, gelsemine solutions were neutral or repellent, in accordance with previous studies (Adler, Irwin, 2005; Gegear et al., 2007).

CONCLUSION

In conclusion, we tested preference for and repellence by four alkaloids in a nectar solution. We randomized the positions of flowers, which prevented the bees from memorizing the position of the preferred nectar. Data suggest that honey bees prefer caffeine not only because it elicits addictive behaviour, but also because of a taste preference. In contrast with other studies, we did not observe a preference for nicotine-containing nectars.

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USE OF MOBILE PIPELINE WITH SELF-REGULATED WATER OUTLETS FOR FURROW IRRIGATION

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Surface irrigation is one of the most common irrigation methods, but it has its own significant drawbacks. A mobile pipeline for furrow irrigation from closed irrigation network was designed and its parameters were proved in order to improve furrow irrigation. The mobile pipeline consists of plastic pipes based on 10 pairs of spring skis connected in the middle by a flexible connection. It is known that water flow into the furrow decreases along the pipeline due to pressure losses along the length of the pipeline. It was proposed to use water outlets ensuring uniform supply of water to each furrow by the presence of the butterfly valve in housing of the water outlet in the mobile pipeline. In order to ensure sustainable valve position, an equation was derived and values of the area of the upper and bottom parts of the valve were obtained. According to the pipeline field test results, water distribution uniformity coefficient through the outlets was 0.98, at a flow rate of 1.0–3.0 $l \cdot s^{-1}$ and hydraulic slopes along the pipeline of 0.001–0.005.

water flow, uniformity of irrigation, mechanization, pipe, butterfly valve

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INTRODUCTION

Irrigated land worldwide is one of the main factors for the stability of agricultural production and ensuring the food security. Irrigated land, constituting less than 20% of arable land, produces more than 40% of crop production (Bruinsma, 2003).

Surface irrigation nowadays is one of the most common irrigation methods, as it has several advantages: low power consumption; simplicity, and high reliability in operation; weak dependence on wind speed; low capital investment in the construction of the irrigation network. However, surface methods have significant drawbacks: low level of mechanization, irrigation efficiency does not exceed 50–70%; significant losses due to surface fault and depth filtration; uneven irrigation (Heermann et al., 1990). Agronomic requirements imposed on the surface irrigation system are the following: uneven supply of water in furrows should not exceed ± 10% of a given norm of watering; simultaneous operation of all pipelines; crop damages should not exceed 0.2%; soil erosion at the joints and pipe irrigation water outlets is not allowed; operation area of 5–30 ha (with different configurations); length of irrigation furrows 100–600 m, depth of 13–16 cm.

Recently developed technologies and facilities partially correct the shortcomings of the traditional surface irrigation, hindering its development. Primarily this includes designed precision layout of field surface using laser equipment (Jat et al., 2006), new ways of irrigated furrow formation (Schwankl et al., 1992; Yonts, 2007), as well as proved technologies and irrigation regimes that enable uniform moisture along the furrow length (Ampas, 1998; Nasseri, 2013). Development of technological schemes of irrigation provides the parameters such as furrow length, water flow to the furrow, irrigation duration according to the given calculated rate with the width of the irrigation's front and the value of head in the irrigation network (Santos, 1996; Horst et al. 2005; Holzapfel et al., 2010). For quality irrigation on a well-designed field, it is necessary to use technical means for supply-

ing water to the field, allowing productivity increase, reducing the seepage loss, ensuring even flow of water in the furrows, and reducing the cost of labour and resources.

Flexible pipes, semi-rigid collapsible pipelines, rigid collapsible steel and aluminum pipes are currently available (Walker, 1989; Zerihun et al., 2001; Nasseri, 2013). However, all these mechanisms are time consuming to assemble and service; and the diameter of the water outlet holes along the length cannot be regulated to change the flow of water to the furrow. To improve the technology of furrow irrigation, there was a need to propose a mobile pipeline enabling a uniform flow of water in furrows, regardless of the total pressure in the network and the slope along the pipeline, and to reduce the cost of labour and resources.

To achieve this purpose, the following tasks must be resolved: to develop a mathematical model of the impact of water flow on the valve turnouts ensuring equal flow of water to furrows; to substantiate the structural and technological scheme and parameters of the irrigation pipe with regulated discharge outlets; to study the influence of the parameters and operating modes of the irrigation pipeline of the quality indicators.

MATERIAL AND METHODS

Mobile irrigation pipes (rigid and flexible) may be used in the planned areas with a hydraulic slope of more than 0.003 in the longitudinal and transverse watering.

For irrigation of crops in the pipeline is provided with two series of outlets: at the bottom by each 70 cm and by 45 cm at the top. Changing of the position to top-bottom if the support of clamps are loosening and installing plugs, that allows us to adjust the distance between the discharge outlets to 45, 70, 90, 135, 140, 180, and 210 cm. Outlets are installed in the lower part of the tube, which promotes quick emptying of the pipeline after water supply.

The length of the pipeline depends on the distance between hydrants closed irrigation network. In this embodiment, the distance between hydrants is 50 m. The choice of pipe diameter depends on the length of the pipeline and conditions of water flow supply. In order to adjust water flow into the furrow, the designed self-regulating outlets are used.

The theoretical work was based on the provisions of law and methods of fluid dynamics using mathematics. In laboratory experiments the theory of hydrodynamic similarity with the Newton's number as the general criterion of hydrodynamic similarity was used.

Investigations were performed in accordance with applicable standards in laboratory and field conditions based on conventional techniques using the theory of mathematical planning and processing of experimental data.

Theoretical substantiation of the design parameters of the pipeline

As given above, we have developed a mobile pipeline consisting of two plastic pipes connected by ameliorative cloth and clamps, based on (through the brackets) spring skis the length of which exceeds two aisles of furrows, several series of water outlets along the length of irrigation pipe, a manometer installed to monitor the pressure, and a connecting bend (Kokurin et al., 2008). One end of the spring ski is rigidly fixed to the bracket, and the other one is placed freely (Fig. 1). Two rows of water outlets are made along the length of pipeline at an angle of 180º through 0.70 and 0.45 m, respectively, while the unused row of holes is closed with plugs. Water is supplied from a hydrant of the closed irrigation network through the connecting bend. Length and diameter of the pipeline are justified depending on the feed network parameters.

The technological process of irrigation using a mobile pipeline is as follows. A tractor moves the irrigation pipe using a rope from one position (irrigated) to another (to be irrigated). For this purpose, the front end of irrigation pipeline is connected to the hydrant via a flexible hose by a clamp. After filling the pipeline with water, its mass increases 7–10 times, and the pipe rests on furrow ridges.

At the end of irrigation of this position, hydrant was shut; flexible connection was removed and secured on irrigation piping.

During this time, irrigation pipe started to be empty, the load on the spring ski decreased and it straightened, raising the irrigation pipe above the soil surface. Irrigation pipeline moved to a new position with the help of the tractor.

Spring ski is a constant cross-section leaf with a constant radius of curvature along the entire length, with one clamped and one sliding supports (Fig. 2).

Based on the condition that the maximum load on the spring ski should correspond to the value of the static spring deflection (Parhilovsky, 1978)

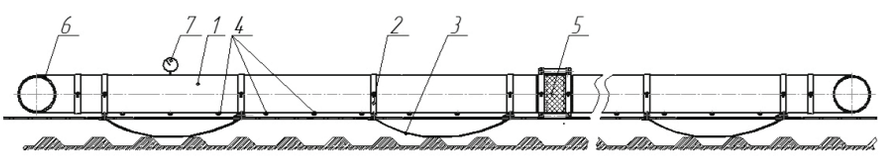

Fig. 1. Layout of irrigation piping

1 = pipe, 2 = clamp, 3 = spring ski, 4 = water outlet, 5 = flexible connection, 6 = connecting bend, 7 = gauge

$$P_T = \frac{4E_y b_0 a_0^3 f}{l_r^3 \delta_P} \quad (1)$$

where:
P_T = applied load (N)
l_r = spring length (m)
E_y = modulus of elasticity (MPa)
b_0 = spring width (m)
a_0 = spring thickness (m)
f = value of static deflection
δ_p = deflection magnification (δ_p = 1)

Total number of spring skis that provide pipeline lift and lowering (P a r h i l o v s k y , 1978)

$$n_p = \frac{\sum Q}{P_T} \quad (2)$$

where:
$\sum Q$ = total load on the spring skis (N)
n_p = number of spring skis in the pipeline

In order to ensure a uniform supply of water to the furrow, and in view of pressure head losses along the length of the water pipe, water outlets are mounted in pipe structure with the butterfly valve of a trough shape (G r u d i e v , V y s o c h k i n a , 2009).

The butterfly valve of a trough shape, consisting of two platforms ($S_в$ and $S_н$), is mounted on the axis in the housing of the water outlet (Fig. 3). Upper platform $S_в$ is in the housing part 1 of the pipeline, and the lower one is in pipeline 2 of the irrigation piping water outlet.

At the time of water supply into the pipeline, water outlet valves are rotated (Position I) and shut the water outlet openings under the action of the pressure force F_1.

After filling the pipeline with water under the action of pressure force on the bottom of valve F_2, simultaneous opening of the water outlet openings (Position II) takes place, wherein the angle of valve inclination depends on the water flow rate.

The water flow through the water outlet depends on cross-sectional area and the flow head (L i k h i , 1995; R a i n k i n a , 2005):

$$Q_i = \mu_p \omega \sqrt{2gH_u} \quad (3)$$

where:
Q_i = water flow through i-th outlet ($m^3 \cdot s^{-1}$)
μ_p = flow coefficient through outlet
ω = cross-sectional area (m^2)
g = acceleration of gravity ($m \cdot s^{-2}$)
H_u = flow head (m)

Preliminary measurements of the flow rate in the pipeline at the working model have shown that the flow regime is turbulent (R >> 2400). Taking into account the rapid increase in the velocity modulus from 0 to V_{max}, it is possible to substitute the actual speed at every point of flow interaction with the valve by its average speed $\bar{V}_i$.

The butterfly valve of the water outlet takes the operating position with equal moments of forces acting on the top and the bottom valve. Along with the rule of moments we used the law of conservation of energy, and its particular case in hydrodynamics – the Bernoulli equation.

Based on the condition of equality of the outlet flow $Q_i = Q_{i-1}$, after equating flows, after transformations (L i k h i , 1995; R a i n k i n a , 2005) we obtain:

$$\sqrt{2gH_{ui}} = \left(1 - \frac{S_н \cos\alpha}{S_в}\right) \sqrt{2gH_{ui} + \frac{\bar{V}_{i-1}^2}{2{,}38}} \quad (4)$$

where:
H_{ui} = pressure head of the flow through the i-th outlet (m)
$S_в$ = area of the top valve platform (m^2)
$S_н$ = area of the bottom valve platform (m^2)
α = angle by which the valve has to decline (allowing forecasting the flow section of the outlet and the flow of water into the furrow)

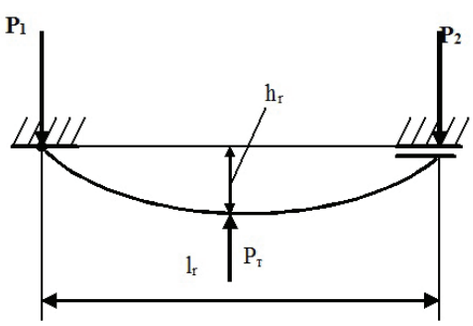

Fig. 2. Calculation scheme of spring ski

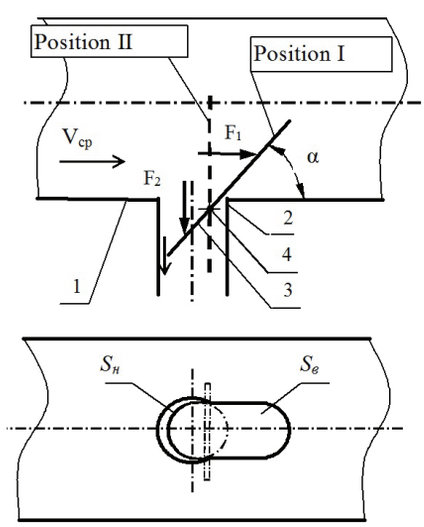

Fig. 3. Scheme and photo of a self-regulating water outlet design
1 = pipeline, 2 = water outlet pipe, 3 = butterfly valve, 4 = axis

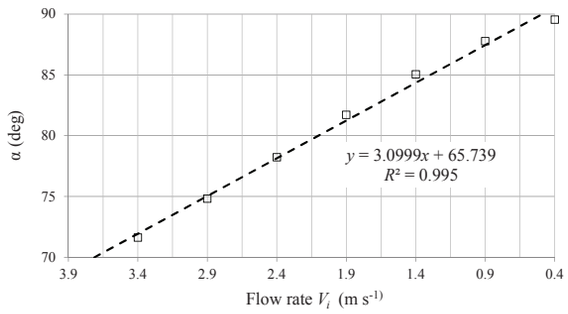

Fig. 4. Dependence of valve angle of inclination on the flow rate

Thus, the equality of outlet flow depends on the area ratio of the upper and lower valves, flow pressure head, flow rate of fluid in the outlet area, and valve inclination angle. Angle α depends on the speed of fluid flow $\alpha = f(V_i)$ (Fig. 4).

A prerequisite for sustainable outlet valve position is the equality of moments applied to its upper and lower parts, $M_в = M_н$.

When determining the forces acting on the body by the water flow, generally used equations expressing the law of change of momentum are differential equations of Navier-Stokes and Reynolds (A l t s c h u l , 1977). Then the moment of force acting on the upper and bottom parts of the valve is determined as follows:

$$M_в = F_{iα} l_в \sin\alpha \tag{5}$$

$$M_н = F_{iн} l_н \cos\alpha \tag{6}$$

The area of the bottom part of the valve is limited by the outlet diameter.

Taking into account equations (4,5,6) and given that the sectional area of the water outlet may be defined as a circular area (diameter equal to the diameter of the passage section), and equating the moments acting on the upper (eq.5) and bottom (eq.6) parts of the valve, after transformation the upper valve part area is:

$$S_в = S_н \sqrt{\frac{2\left(\left(1+k^2\right)gH_u + \frac{2}{3}gl_H \sin\alpha + \frac{k(n+1-i)\overline{V}_n}{1{,}19}\cdot\sqrt{2gH_u} + \frac{(n+1-i)^2\overline{V}_n^2}{2{,}38}\right)\beta\ ctg\alpha}{(n+1-i)\overline{V}_n^2}} \tag{7}$$

where:

β = coefficient taking into account the configuration of the valve

Since the relationship between the mean flow rates at the outlets is recurrent, the resulting expression is valid for any sequence number of the outlet.

Laboratory experiments

The theory of hydrodynamic similarity (B e r t r a m , 2011) was used in laboratory experiments. The water flow rate and the flow rate along the pipeline were measured by ultrasonic flow meter (industrial ultrasonic flow meter «Vzljot PR», association «Vzljot», St. Petersburg, Russian Federation). To measure the flow and flow rate in the pipeline, flow meter sensors were mounted before and after each outlet (Fig. 5) alternately in ascending order. By changing the pressure in the pipeline, controlling reading on the gauge, measured parameters were indicated on the flow meter display with the selected dimension and display period.

The experiment results revealed that when water is supplied to the irrigation pipeline, the water outlet openings are completely shut under the influence of pressure force F_1.

When flow rate is steady, water flow rate at the beginning of the pipeline is greater than at its end, and the valve tends to close the initial water outlet hole to a greater extent than the next ones, depending on the distribution of water pressure along the length of the pipeline (Fig. 6).

Equality of the outlet flow depends on the area ratio of the upper and bottom valves, flow pressure head, flow rate of fluid in the outlet area, and valve inclination angle $\alpha_1 < \alpha_2 < \alpha_3$.

Field tests were conducted in the Stavropol Territory, Russian Federation, in 2008–2009 during furrow irrigation of corn. The furrows were located crosswise of piping arrangement and hydrants line. Irrigated land with well levelled surface was chosen, irrigation furrows were 200 m, and hydraulic slope was from 0.003 to 0.01.

The distance between hydrants was 110 m, hydrant diameter was 320 mm. The maximum capacity of the hydrant of the closed irrigation network was 210 l·s^{-1}, the flow into the furrow was up to 3.0 l·s^{-1}.

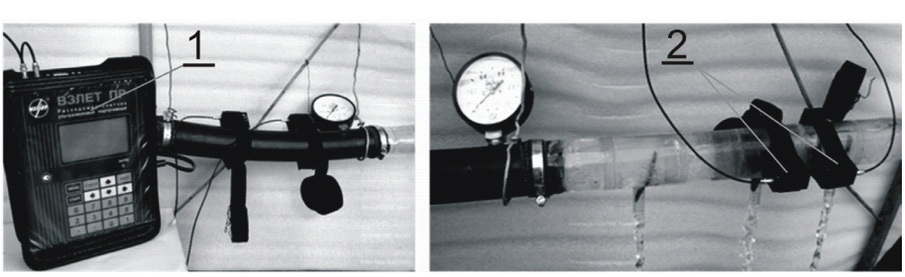

Fig. 5. Experimental mount to determine the flow of water by outlets 1 = ultrasonic flow meter, 2 = flow meter sensors

Table 1. Water pressure in the pipeline at a total flow rate of 140 l s^{-1}

On the length section of pipeline (m)	Water pressure in the pipe (m of water column)	
	with a slope of i = 0.001	with a slope of i = 0.005
10	6.02	6.00
20	5.40	5.10
30	4.75	4.43
40	4.30	3.94
50	4.05	3.61

The outlet diameter (30 mm) was selected based on the maximum flow to a furrow through the last water outlet; water flow through the outlet was adjusted from 1.0 to 3.0 l·s^{-1}. The area of the upper part of the valve was $7.54 \cdot 10^{-3}$ m^2, of the bottom one $5.77 \cdot 10^{-3}$ m^2.

Studies were carried out at a total flow rate of the water in the pipe 100, 140, 180 l·s^{-1} at hydraulic slopes 0.001 and 0.005.

RESULTS

The head loss by the length of the pipe was determined along the pipeline on a hydraulic slope from 0.001 to 0.005 at total water flow rate of 140 l·s^{-1} (Table 1). Head loss along the pipeline increased with slope decreasing.

Water flow in a furrow was measured through the water outlet holes and self-regulated water outlets. Deviation of water flow along the length of irrigation pipeline was ± 0.02 l·s^{-1}, with a total flow rate of 140 l·s^{-1}, which meets the agronomical requirements. A change in the slope of the field along the pipeline did not affect the flow at outlets – flow remained constant. This suggests that the design of the water outlets operates through internal hydraulic processes, regardless of the slope along the pipeline. Hydraulic processes are based on interdependent speed fluid flows affecting butterfly valves of outlets (Fig. 7).

It has been determined that the butterfly valve of the outlets damps flow energy in the pipeline, reducing the energy of the jet fed into the furrow, thereby decreasing the depth and diameter of the plunge basin in the water discharge area (Fig. 8).

The total water pressure drop along the pipeline was 4.4 m. Generated water pressure at hydrant of water column of 5.4 m ensured the total flow of 140 l·s^{-1}. The coefficient of land use of irrigated area with the furrow length of 200 m was 0.96.

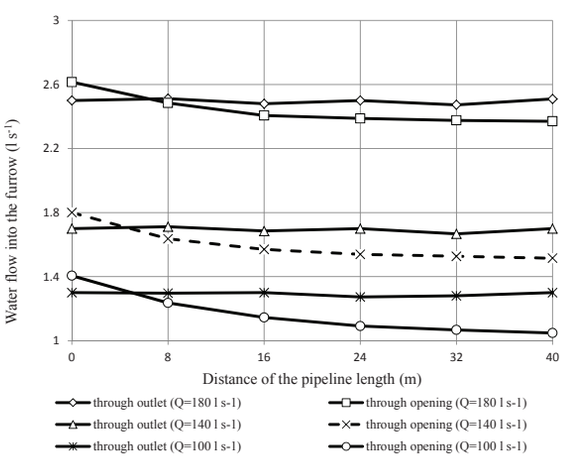

Fig. 7. Water flow in the furrow along the pipeline with a slope of 0.005

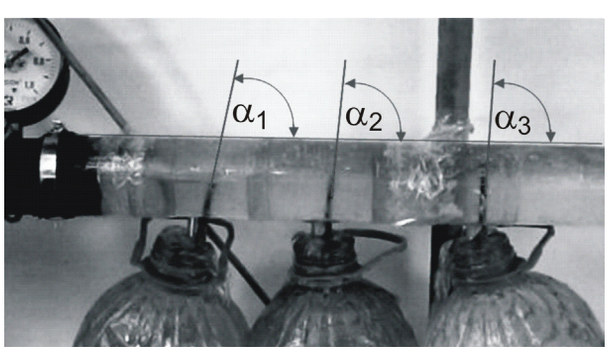

Fig. 6. Position of the water outlet valve in the steady flow of water in the pipeline

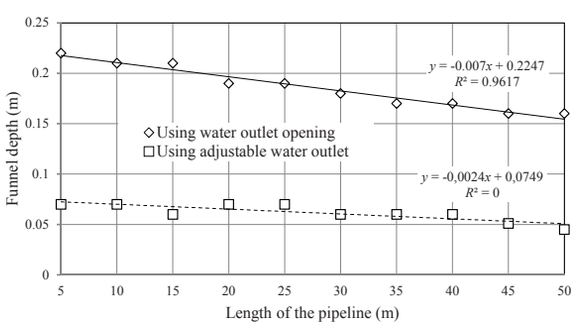

Fig. 8. Depth of plunge basin in water discharge area

DISCUSSION

Pipelines for furrow irrigation currently have turnouts outlet with constant flow rate (Walker, 2003) or different diameter along the length of the pipeline (Enciso, Peries, 2005).

Outfalls of constant cross-section do not provide the same flow along the length of the pipeline due to pressure losses (Bertram, 2011). Choice of different water outlets diameters is connected to the complexity of the calculations. With a constant cross-section it is not possible to change the water flow quickly under varying soil climatic conditions.

In many countries, various surface irrigation design of ground irrigation pipes is used. Most pipelines are equipped with automatic control sensors (Walker, 1989; Sharma, Sharma, 2008). Using pipes with risers-water outlets and electronic control unit irrigation worsens conditions for unimpeded cultivation of row crops and increases the cost of labour and funds for the building of irrigation networks.

Using the laws of hydrodynamics, we have determined the size of the dampers (Kokurin et al., 2008; Grudiev, Vysochkina, 2009) providing self-regulating of the water flow along the pipe, regardless of the slope along the pipeline and created pressure on the network.

It was established that the use of pipelines for supplying water to furrows can increase the coefficient of water use, but it does not provide a uniform feeding of water in furrows. The regularities of the movement of water in the pipe in view of mode - turbulence in the hydraulically smooth pipes, allows estimating the head loss along the pipe in continuous distribution of water in furrows, depending on the length and diameter of the pipe, slope and water head in the hydrants.

CONCLUSION

When watering crops, it is necessary to provide greater water flow than it is set on the hydrant water pressure considering head losses along the pipeline equal to water flow through all the outlets, and reduction of erosive flow rate at the exit from outlets.

The designed parameters of water outflow were proved to reduce the rate of water discharge and define flow. Taking into account flow equality in each outlet, the dependence of valve angle on the flow rate along the length of the pipeline was deduced. Taking into account the conditions for sustainable position of outlet's valve, i.e. equality of moments applied to the upper and bottom parts of the valve, the sizes of the upper and bottom parts of the valve were determined. The mathematical model of the impact of fluid flow on the valve turnouts ensured uniform water flow in furrows and justified the design parameters of water outlets with a diameter of 30 mm, equipped with butterfly valves, the upper part ($7.54 \cdot 10^{-3}$ m^2) of which located in the cavity of the pipeline, and the lower one ($5.77 \cdot 10^{-3}$ m^2) located in the overflow tube, blocking the flow area depending on the pressure, and the expiration of providing water supply to the furrow with a coefficient of uniformity of 0.98 at a flow rate of 1.0–3.0 l·s^{-1} and with the slopes along the pipeline 0.001–0.005.

It was found that the coefficient of land use of irrigated area with furrows length of 200 m located according the transverse pattern of location for the pipeline operation from the closed irrigation network was 0.96, which does not exceed the statutory value.

It was established that the butterfly valve of water outlet reduces the energy of flow in the pipeline, reducing the energy of the jet supplied to the furrow, and as a result the depth of the erosion craterwill be reduced by half, as well as its diameter.

The use of irrigation piping with adjustable outlets can improve the efficiency of water use by 12%, uniformity of water flow into the furrows by 9%, greatly facilitating the work of irrigators, and allows reducing the cost of manual labour.

The designed irrigation pipeline with self-regulating outfalls can be used for irrigation of cultivated crops in furrows at any cross slope areas (within tolerance) with equal flow dosing into a furrow.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS:

P_T = applied load (N), l_r = spring length (m), E_y = modulus of elasticity (MPa), b_0 = spring width (m), a_0 = spring thickness (m), f = value of static deflection, δ_p = deflection magnification, n_p = number of spring skis in pipeline, ΣQ = total load on spring skis (N), Q_i = water flow through the i-th outlet ($m^3 \cdot s^{-1}$), μ_p = flow coefficient through outlet, ω = cross-sectional area (m^2), g = acceleration of gravity ($m \cdot s^{-2}$), H_u = flow head (m), H_{ui} = pressure head of flow through the i-th outlet (m), $S_в$ = area of top valve platform (m^2), $S_н$ = area of bottom valve platform (m^2), α = angle by which valve has to decline, allowing forecasting flow section of outlet (deg), F_1 = pressure force on upper parts of valve (N), F_2 = pressure force on bottom parts of valve (N), $\overline{V_i}$ = average speed ($m \cdot s^{-1}$), M_B = moment of force acting on upper parts of valve (N·m), M_H = moment of force acting on bottom parts of valve (N·m), b = coefficient taking into account configuration of valve, k = rate coefficient (equal to flow coefficient), V_n = average flow rate at the i-th outlet zone ($m \cdot s^{-1}$),$F_{i\alpha}$ = normal pressure force on upper parts of valve at the i-th outlet (N),F_{iH} = normal pressure force on bottom parts of valve at the i-th outlet (N), l_B = length of upper part of valve (m), l_H = length of bottom part of valve (m).

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INFLUENCE OF GLASS POWDER SIZE SORTING ON PROPERTIES OF COMPOSITE SYSTEMS*

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Glass powder ranks among the secondary raw materials, which can be used in the interaction with polymeric materials. In the present experiment the polymeric/epoxy particle composite with different sizes of glass powder particles is described. Such utilization of recyclable materials is environmental-friendly and should be preferred. The size of particles forming the filler of the described composites is one of the key characteristics affecting the mechanical properties. Due to the properties of the systems filled with glass powder, these materials can potentially be used in agriculture (renovations, adhesive bonding, cementing, etc.). In the experiment, glass powder was dimensionally sorted through sieves. Three fractions of glass powder with particles size of 0–30, 30–50, and 50–90 μm were created and utilized, the average particle size being 18.7, 38.7, and 72.6 μm, respectively. The interaction of the 18.7 μm particles did not lead to a statistically significant decrease of shear strength values in the interval 0–20 vol.%. The presence of glass powder, however, in all cases decreased tensile strength.

adhesion; agriculture; cohesion; epoxy resin; glass powder

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INTRODUCTION

Epoxy resins are polymeric materials with macromolecular chains which contain more than one epoxy group, i.e. oxirane or ethylene oxide group. This group of structural adhesives is often used in agriculture – either as adhesives or composite systems (Kejval, Müller, 2013; Müller, Herák, 2013). Epoxy resins with a dispersed particulate filler are mostly cured by polyamines while forming three-dimensional networks (in most cases without the excess pressure). This curing process is carried under normal (laboratory) temperature (Mleziva, 1993; Petrie, 2006). Particles have multiple functions in reaction resins. Particles influence (in some cases optimize) mechanical properties of resin and last but not least reduce costs. The resulting mechanical properties are given by a number of parameters, the most important being viscosity of resin, wetting of the filler with resin, nature of the linkages between resin and particles, etc. (Milton, 2002; Valášek, Müller, 2013a).

The described experiment is primarily devoted to the basic mechanical characteristics of epoxy resin filled with glass powder (GP), i.e. adhesion and cohesion. Adhesion is reflected on interfaces of both, the filler and resin, and the composite system and adherend to which the system is applied. Adhesion between the filler and the used resin is affected, among others, by morphology of particles and their size. Conclusions by Cheng et al. (2002) point to the fact that roughness of particles ranks among the most important parameters affecting adhesion of particles to matrix. Experiments performed by Zhai et al. (2006) also show the influence of particles on the interface of the filled adherend and the system, where particles (particles of Al_2O_3, $CaCO_3$, SiO_2 were used in the experiment) can improve adhesiveness (adhesion) of epoxide to steel. From the viewpoint of cohesion

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(cohesiveness of a filled system), Nakamura et al. (1992) described a crucial influence of particle size on strength and stated a decrease in tensile strength of polymers with increasing particle size (6–42 µm). Hojo et al. (1974) formulated similar conclusions on the epoxy resin strength decrease with the increasing particle size by a mathematical relationship

$$\sigma_k = \sigma_m + k_p(V_p)d_p^{-1/2} \quad (1)$$

where:
σ_k = composite breaking strength (MPa)
σ_m = matrix breaking strength (MPa)
$k_p(V_p)$ = constant as a function of the volume fraction of particles (-)
d_p = average particle size (mm)

The aim of the experiment is to describe the adhesion of epoxy filled with different GP particle sizes to steel adherend (this type of material is commonly used in agriculture) by means of shear strength. Cohesion is described by tensile strength. Minimization of the composite system price through the inclusion of a secondary raw material – glass powder – is also one of the important issues dealt with in the present contribution. Effective handling of recyclable materials reduces the burden on the environment, costs, and saves primary raw materials.

MATERIAL AND METHODS

Matrix and filler

Filling reactive resins with a particulate filler is performed during the uncured state, depending on the required resulting properties. Preparation of filling mixtures is crucial with respect to the amount of pores contained in the material after curing. In experimental programs mixing with or without the use of vacuum can be applied. The resulting shape is then achieved, for example, by following casting into moulds with zero adhesion to moulded mixture or the system is applied directly to the adherend. After reaching the final shape the resin is cured according to the technological requirements. A two-component epoxy resin (Epoxy) Glue Epoxy Rapid F (DCH-Sincolor a.s., Karlovy Vary, Czech Republic) (density 1.00 g·cm^{-3}) was used in the experiment. This epoxide exhibits high cure rate (9–10 min at 50 g of stiffed mixture, 23°C) and reduced sagging of the surfaces, glass transition temperature 59°C.

The resin was filled with GP (density 2.50 g·cm^{-3}) made from recycled glass shards (classified as waste 20 01 02 – Glass according to the Waste catalogue of the Czech Republic), which are processed by the company AMT Příbram, s.r.o. Glass shards are pulverized by a ball mill and this secondary raw material is then sieved by rotating sieve. The GP used (particles less than 90 µm in size) is traded on the Czech market by Refaglass, a.s. The purchased GP was dried in the drying room (humidity lower than 1%) and then sieved using hand sieves with mesh sizes of 50 and 30 µm (Fig. 1) to prepare different fractions of particle sizes. The different fractions were sizing 0–30 µm, 30–50 µm, and 50–90 µm.

The composite mixture which was cured according to the manufacturer's requirement (at room temperature of 24 ± 2°C, humidity 40–50%) was prepared by mechanical mixing of GP and epoxy composite. The filler concentration was expressed by volume percentage and corresponded to 1, 2, 3, 4, 5, 10, and 20%. The test specimens for testing the tensile strength were cast in moulds made of two-component silicone rubber in accordance with the standard ČSN EN ISO 3167 (Plastics – Multipurpose test specimens). Tensile strength values describing the cohesive strength of the cast specimens were measured in accordance with ČSN ISO 527-1 (Plastics – Determination of tensile properties, Part 1: General principles) always on 6 specimens appropriate to the standard. The test was carried out on a universal testing machine LabTest 5.50ST (Labortech s.r.o., Opava, Czech Republic) (50 kN). Test speed (crosshead movement) corresponded to 6 mm·min^{-1}. Hardness of the specimens was evaluated using Shore D scale (ČSN EN ISO 868).

Shear strength (adhesion to steel adherend) was determined in accordance with the standard ČSN EN 1465 (Adhesives – Determination of tensile shear strength of rigid adherends on overlapped specimens). Based on recommendations of Teng et al. (2011) that the most appropriate treatment of adherends before the application of epoxy resins includes blasting, the surface of the adherend was blasted with corundum before applying the system (F80 at 90°C). After the blasting process, the surface of adherends was degreased and cleaned with perchlorethylen. The surface treatment of materials has been stated as crucial in many applications (Novák, 2011; D'Amato

Fig. 1. Sieves used for glass powder particles size sorting into individual fractions

Table 1. Theoretical density and porosity glass powder (GP)/epoxy

GP (vol.%)	Theoretical density (g·cm^{-3})
1	1.01
2	1.03
3	1.05
4	1.06
5	1.08
10	1.15
20	1.30

et al., 2014; Rudawska, 2014; Affatato et al., 2015). As important factors describing the surface integrity were selected roughness parameters, which were determined by the profile according to ČSN EN ISO 4287 using a profilometer Surfest 301 (Mitutoyo America Corporation, Aurora, USA). Wavelength (cut-off) corresponded to 0.8 mm. During the cure process joints were loaded with 230 g·cm^{-2}. Thickness of the resin layer (gap) between adherends was evaluated using a stereoscopic microscope (SZP 11-T Zoom, Arsenal s.r.o., Prague, Czech Republic) via embedded software (Quick Photo Industry). GP and test specimens for each experiment performed are shown in Fig. 2.

The obtained data sets were evaluated by statistical methods, which were intended to indicate statistically significant changes from the perspective of the measured mean values of these sets.

RESULTS

Values for theoretical density, one of the basic characteristics of GP/epoxy composites, are shown in Table 1. They are based on the theoretical densities of resin and GP – perfect wetting of the filler by epoxy is considered in the calculation.

Fig. 2. Glass powder/epoxy specimen for shear strength test (left), specimen for a tensile strength test (right)

GP was sieved on a production line in fractions with particle size below 90 µm. This particle size represented the highest level of assessed intervals. GP particles were classified according to their size and each interval of particle size distribution was subsequently subjected to optical analysis on a stereoscopic microscope. Then the average size of particles was determined for the individual intervals – see histograms in Fig. 3. For each interval, the mean particle size corresponded to 18.7 ± 7.7, 38.7 ± 7.2, and 72.6 ± 16.6 µm.

The presence of GP in all cases increased hardness (Fig. 4). Unfilled resins evinced Shore D hardness of 89.45 ± 1.25. Hardness was the highest at 20% GP concentrations. The highest mean value of

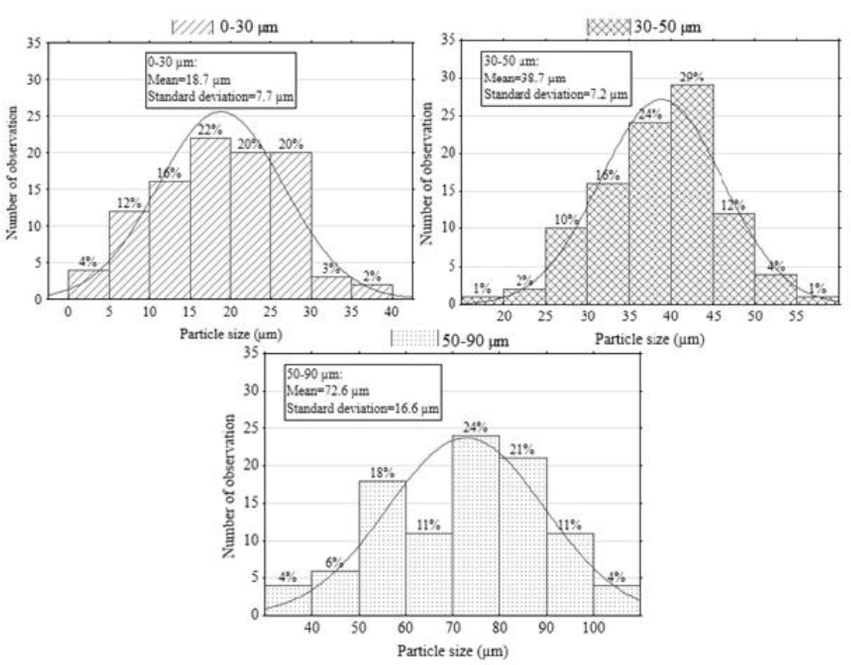
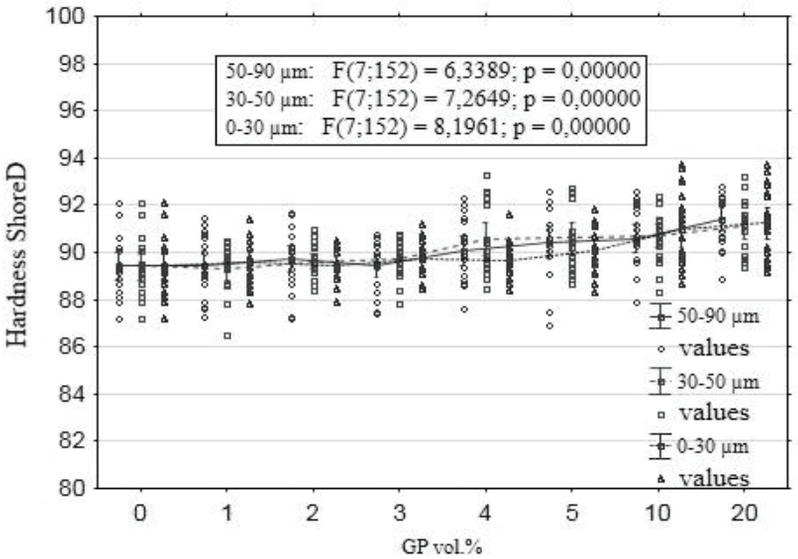


Fig. 3. Dimensional analysis of glass powder particles sorted into individual fractions

Fig. 4. Hardness of glass powder/epoxy composites with different particles size



Shore D hardness was measured at 20% concentration (50–90 µm), i.e. 91.38 ± 01.03. Coefficient of variation of measurements ranged 1.2–2.0%.

The impact of different particle sizes on the adhesive properties of the composite mixture was evaluated on steel adherend through shear strength. Prior to the system application on a surface, the surface parameters should be assessed. From this viewpoint basic roughness parameters Ra and Rz are particularly relevant (Fig. 5).

Unfilled resin exhibited shear strength of 11.49 ± 0.91 MPa (coefficient of variation was 7.9%) (Fig. 6).

In all cases except for 0–30 µm/1% (an increase of 0.5% to 11.54 MPa) a slight decrease in shear strength values occurred due to the inclusion of the filler. The lowest measured shear strength was 9.48 ± 0.59 MPa (50–90 µm/3%, a decrease of 17.5%, coefficient of variation 5.9%). As is visible from the statistical analysis (Table 2), in the case of decreasing values of shear strength, this decrease can be discussed as statistically insignificant (due to the measured standard deviations), where the coefficient of variation of the measuring reached 15.8%. Only in the interval with particle size 0–30 µm the filled resin systems comparing to the unfilled resin systems do not evince any statistically significant decrease (hypothesis H_0) in shear strength ($P > 0.05$). In other cases, this statement is not fulfilled.

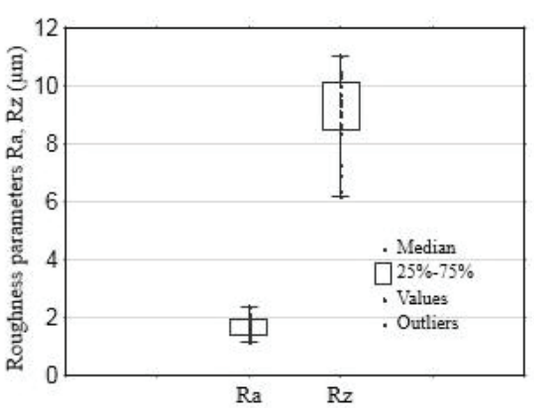


Fig. 5. Roughness parameters of steel adherends (S235J0) in the place of a joint

The analysis of failure type in the place of the joint was carried out by a stereoscopic microscope after testing according to ČSN ISO 10365 – Adhesives – Designation of main failure patterns. In all cases (concentration and particle size of GP) the failure was in the adhesive type (Fig. 7).

The dependence of cohesive characteristics on different particle sizes of GP was evaluated by tensile strength (Fig. 8). Unfilled resin reached tensile strength of 42.56 ± 2.64 MPa (coefficient of variation 6.2%).

In all cases the inclusion of GP particles resulted in a statistically significant decrease of tensile strength values of the systems. Higher concentrations of the filler led to a greater decrease of the value. The lowest value of –27.30 ± 2.86 MPa (coefficient of variation 10.5%) was measured in the system 50–90 µm/20%.

DISCUSSION

In terms of adhesion, we may partially agree with the conclusions of Z h a i et al. (2006), who describe the positive effect of particles on the adhesion of epoxy resin to the steel adherend, the optimum concentration of Al_2O_3 particles (60–100 nm) indicated by the authors was 2 wt.% (increase in strength from 3.6 to 7.8 MPa). The increase in adhesion was observed in only one case (0–30 µm/1%, an increase of 0.5%) and this increase was not statistically significant (see results of *T*-test). It should, however, be noted that this experiment describes the particle size in the order of

Table 2. *T*-test: statistical comparison of shear strength (0 : 1, 2, 3, 4, 5, 10, 20%)

T-test H_0: ($P > 0.05$)	Glass powder (vol.%)						
Particle size (μm)	1	2	3	4	5	10	20
50–90	0.06	0.01	0.00	0.18	0.06	0.30	0.19
30–50	0.41	0.03	0.29	0.08	0.01	0.15	0.02
0–30	0.93	0.84	0.53	0.84	0.40	0.20	0.06

Table 3. *T*-test: statistical comparison of tensile strength (0 : 1, 2, 3, 4, 5, 10, 20%)

T-test H_0: ($P > 0.05$)	Glass powder (vol.%)						
Particle size (μm)	1	2	3	4	5	10	20
50–90	0.01	0.01	0.01	0.01	0.00	0.00	0.00
30–50	0.01	0.00	0.00	0.00	0.01	0.00	0.00
0–30	0.01	0.00	0.01	0.00	0.01	0.00	0.00

tens of micrometres, comparing to results with particle size in nanometres. We may partially agree with the results of Dekkers, Heikens (1983), stating that the inclusion of glass spheres leads to good adhesion to the metal adherend – the authors describe a cohesive kind of disruption on aluminium adherends. The adhesive failure dominated in the experiment carried out on steel adherend. This shows that the weak point on the interface is the interaction. For example, the optimum surface treatment of adherend could lead to an increase in strength at this interface.

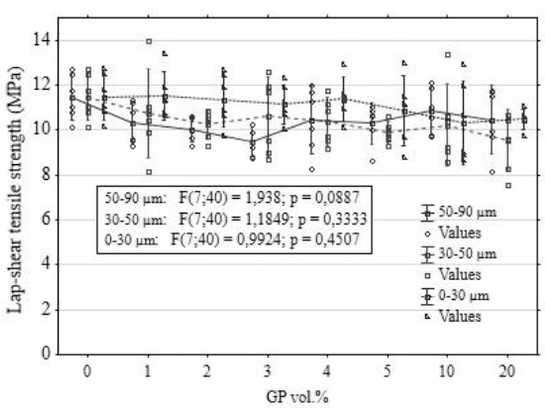


Fig. 6. Lap-shear tensile strength of glass powder/epoxy composites with different particles size

In terms of tensile strength it is not possible to agree with the conclusions of Kahramana et al. (2008), who describe an increase in aluminium particles strength in the order of tens of micrometres. The inclusion of GP has always caused a decrease in tensile strength irrespective of the used particles size.

The experiment also did not confirm the increase in tensile strength by the inclusion of GP at a low concentration of 5% described by Ku et al. (2010) and Ku, Wong (2012). The decrease in tensile strength can be caused by high porosity of the mixture, which originated only by moulding without the use of vacuum. The preparation is inexpensive and is frequently used in practice. This procedure can minimize the formation of air bubbles, which can then initiate the cracks formation. A decrease in tensile strength of thermoplastics due to the presence of glass sphere was described e.g. by Nicolais, Nicodemo (1974), the strength of the unfilled matrix was always higher than the strength of the filled matrix; this fact was attributed to imperfect adhesion between the phases.

When applying GP into reactive resins or to other adhesives, their properties must be respected (Valášek, Müller, 2013b). For example, some epoxy resins differ in molecular weight and viscosity, which affects the preparation mixture and subsequent interaction between the resin and the filler (particles).

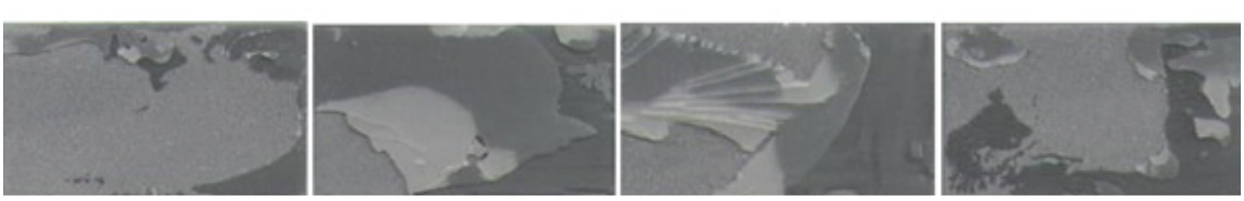
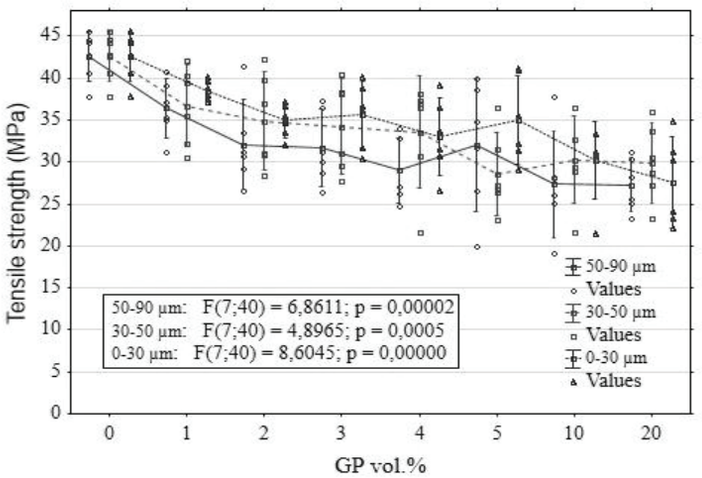


Fig. 7. Typical adhesive type of failure of test specimens (30-50 μm - left: 1%, 2%, 5%, 20%)

Fig. 8. Strength of glass powder/epoxy composites with different particles size



CONCLUSION

Inclusion of GP into reactive resins and adhesives is a method of utilization of secondary raw materials, which is inexpensive and sensitive to the environment. From this perspective, such a use of secondary raw materials should be preferred. Noteworthy results obtained in the experiments can be summarized as follows:

• The presence of GP increased Shore D hardness by up to 2.15%.

• The influence of GP particles size on shear strength was proved: a smaller particles size of 0–30 µm did not lead to a statistically significant decrease in the values of tensile strength.

• The inclusion of GP led to a decrease in tensile strength by up to 35.9%. A clear influence of particle size on tensile strength was not proved in the monitored intervals.

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SUSTAINABILITY OF THE PUBLIC WATER SUPPLY AND SEWERAGE SERVICES OPERATING SYSTEM: A CASE STUDY ON THE EXAMPLE OF THE CZECH REPUBLIC*

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Water services, as a necessity for natural ecosystem functions and a key output from public governance, play a crucial role in forming sustainable relationships between natural, economic, and social factors in the development of society. Primarily, these relationships relate to the natural impacts of weather and climate on the variability of the hydrological cycle. Secondary relationships exist between providers and consumers of the services. Services provided by operators of public water supply and sewerage systems are a specific segment of water services. Their sustainability is controlled on the one hand by public regulation and and on the other by a combination of economic, social, and environmental objectives and the means by which they are achieved. The aim of this paper is, based on the parameters of supply and demand, to quantify the most important aspects of sustainable management of water supply and sanitation enterprises in connection with the current model for state regulation. The methodology is based on an examination of consumer behaviour indicators which can be interpreted from 'water bills'. The comparison of household expenditure on water services in the Czech Republic shows that some are already approaching, and even exceeding, the limit of what is considered social acceptability.

water services; sustainable management; economic regulation; social acceptability; water price

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INTRODUCTION

Water services play a key role in ensuring sustainable development of society. In recent decades, impacts which increase the uncertainty of the sustainability of these services have been strengthening (Zhou et al., 2010; Katko et al., 2012). Primarily, these are the impacts of climate change on the variability of the hydrological cycle (Frederick, Major, 1997; Slavíková et al., 2013), which emphasizes the importance of water accumulation, energy use of water treatment/distribution, water quality, flood and drought prevention, and sustaining ecosystem services. This is reflected in the growth of public expectation that drinking water supply, wastewater removal, and sewerage treatment will all be provided at a reasonable (affordable) price. These services, a subset of water services, are provided by organizations which specialize in public water supply and sanitation. In the Czech Republic, this set of specialists is made up of owners of technical infrastructure (municipalities) and operators who provide water for drinking and the removal of waste water. The operators are public interest enterprises (often privately owned and profit based) and undertake their operations within a framework of environmental, economic, and social objectives set out in law. Sustainable management of these enterprises is related to a complex set of specific economic (natural constraints and technical monopoly), social (focused on public health and safety), and environmental (pollu-

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tion prevention and internalization) constraints which determine costs and revenues, and which influence each other (Del Borghi et al., 2013). The scope of relevant sustainable development issues in this area is evident, *inter alia*, from the Global Reporting Initiative methodology indicators (GRI, 2013).

A major influence on the sustainable management of water supply and sanitation enterprises is – under European rules – the regulation of economic activity and entrepreneurship in the field of public services (in the EU – services in general interest). Public water supply and sanitation services are characterized by economic specificities such as: local monopoly (natural monopoly based on the source of water, technical monopoly based on the use of unique infrastructure), public subsidies/grants (with a link to the public nature of services), and strong links in the social, environmental, and safety areas (Lenton et al., 2008). These enterprises operate in an environment where two principles collide and create tension: the functioning of water supply and sanitation systems in real market environment (resources purchased including labour, consumable materials, energy and infrastructure investment) and the regulation of economic and ecological behaviours and the distribution of social and other public benefits (François et al., 2010). Regulation affects all areas. Public services are largely operated by private companies with exclusive rights for a limited time period and a well-defined geographical space (Boag, McDonald, 2010). This is the result of the process of partial privatization of the water management sector in the Czech Republic in the 1990s. The analyses can therefore be based on data, procedures, and methods common for enterprises on the one hand, and on the other on reference economic regulation, ecological and social functions which combine to directly enforce its extension to a sustainable development framework.

The aim of this paper is, based on the parameters of supply (quantity, price) and demand (consumption, acceptability), to quantify the most important aspects of sustainable management of water supply and sanitation enterprises in connection with the current way of the state regulation. The paper describes the trend of prices of water (followed by sewage) tariffs, the impact on drinking water consumption, and the impact on different population groups. The opportunity for a change in regulation, in the case of the Czech Republic, is also outlined.

MATERIAL AND METHODS

A steady decrease in the consumption of domestic water supplies draws the attention of enterprises because it directly impacts on the economy of operation, costs and revenues, the affordability of innovation activities (including infrastructure upgrades and new process technologies), and the financing of facilities. There appear to be difficulties in public cost recovery and private profitability (Del Borghi et al., 2013). Raising prices for consumers has become a major and effective tool in addressing these problems (OECD, 2010; Wang, Segarra, 2011). Other options for improving enterprise finances (leakage reduction, general cost management) are already partially depleted (progressive residual loss reduction measures tend to be more costly as the 'quick fixes' are attended to first) and other options are constrained by the conditions set by the regulatory environment (the method of cost-based pricing according water law, tenders, grants/subsidies). The urgency of addressing this issue in economic terms can be illustrated by examination of the relationship between the price of water supplied and the income of consumers' demand and their sensitivity to price changes. The first relationship is sometimes referred to as 'social acceptability' of water price (Wang et al., 2010) and is defined by the proportion of households where expenditure on water services exceeds 2–3% of net household income (Hung, Chie, 2013). The latter – income elasticity of demand – characterizes active consumer behaviour and in a specific case it can be interpreted in the context of the theory of demand (McEachern, 2010). Assuming that the quality of supplied drinking water is similar throughout the Czech Republic, then the main indicator of social and other effects is the quantity consumed. Another prerequisite for an investigation of consumer behaviour is transparency of payments (billing according to actual consumption), which is not always a matter of course (Wallsten, Kosec, 2005). The relationship between the amount of water consumed and the social effect is not simple. From a certain level, however, it may pose a more serious problem than 'cost management' and with further general decrease in consumption the social effect may escalate (OECD, 2011).

Regarding environmental aspects, water services enterprises are governed by the laws regulating water abstraction, quality of water from the tap and wastewater discharges in terms of quantity and quality. It can only be stated that the trend of water and sewage tariffs has a positive effect on the abstraction of water as a natural resource, i.e. that the amount of water abstracted for consumption shows a long-term decrease per head of population. This fact, however, has significant implications in the above-mentioned social area (Hung, Chie, 2013), and these are dealt with in this paper.

The general methodology is based on indicators of consumer behaviour (households) as reflected in the water bills (OECD, 2011). Payments for drinking water, including sanitation, are one of the items studied by social statistics in a sample set of respondents. These indicators are compared with the average values of the total amount of billed potable water and

payments for water and sewage tariffs in the Czech Republic as a whole.

The paper builds on an analysis based on Czech Statistical Office (CZSO) data sets: Water Supply and Sewerage Systems (time series 2005–2012), Household Budget Survey and Household Income and Living Conditions (time series 2005–2012) (data broken down by CZSO household definition and the EU or OECD standard), and average prices of the water services at the Ministry of Agriculture of the Czech Republic (MoA) and T.G. Masaryk Water Research Institute (MWRI) data. Basic statistical data and their economic interpretation allow the specification of social stratification in consumption of drinking water, which has a technical dimension (the effect of demographic development on technical capacity and efficient operations), an economic dimension (the impact on cost recovery and economic stability of both owners – municipalities and operators), a social dimension (the direct effect of affordable water services on the quality of life), and a political dimension (the equality in access to water services is limited by price). This is not a transient detail, but an essential characteristic of a broader issue.

As for the previous situation, this one can be indicated by several ways. The basic indicators include the quantification of the specific consumption of drinking water (per person per day). Using the economic analytical apparatus it is possible to determine the response of consumption to price and its sensitivity (Klaiber et al., 2014). If we intend to use 'price' as an instrument in 'demand management', it is necessary to identify as best as possible the relationship between domestic consumption and benefits derived from water and the point at which this is optimized so that the development in the water services sector does not show a tendency to create a social problem.

From the 1990s, water and sewage tariffs in the Czech Republic have been steadily rising and are accompanied by a declining per capita consumption of public water supply and sanitation services. This is not exceptional. In developed countries with more than 100-year-long tradition of public water supply systems, this trend has been evident for decades. In comparison with other European countries and the OECD average, however, water consumption per household member in the Czech Republic is lower (OECD, 2011). The main driving forces for rising prices of water and sewage services are generational replacements of technologies (Kallis, 2010) and basic infrastructure along with increased mandatory technical standards in the development of extensive networks. Implementation of new generations of technologies and materials used to reduce losses and waste is also a driver in price increases. Another influence comes from the attempts to liberalize and privatize the sector (Bel, Warner, 2008), where accompanying negatives have so far been only partially remedied by regulation and re-communalization. Specifically, in the post-communist countries, this price signal has a significant influence on consumer behaviour (Schleich, Hillenbrand, 2009).

The theory of demand derives a change (decrease) of quantity in dependence on the growth of price from marginal utility (Klaiber et al., 2014). Thus, consumption decreases with the growth of prices due to setting prices to the level of full costs and due to the growth of input prices. Water is given as an example of the necessity, the demand for which little depends on the price. Demand is inelastic (Wang, Segarra, 2011). In applying these principles to the case of domestic water supplies we encounter several problems. It is a service which is strongly linked to the subsequent disposal of wastewater, within which the consumer pays for the pollution. Supply of domestic water and sanitation is associated with housing (OECD, 2011). The costs of water and sewage tariffs are therefore strongly linked with housing costs. Domestic water supplied by a local operator – where there is natural and technical monopoly – has no possibility of substitution (Sibly, Tooth, 2008). Due to the strict regulation of water quality standards the quality standard is practically the same for all suppliers (in the Czech Republic). Supply cannot significantly increase or decrease the level of technical equipment required, offer assortment or lower quality for a lower price and *vice versa*. Finally, water for drinking is a necessity, but qualitatively a seemingly homogeneous supply is comprised of some entirely essential parts (drinking consumption, basic hygiene) and parts related to household equipment (dishwasher, swimming pool, gardening etc.). The essential part is seemingly very little elastic (Wang, Segarra, 2011) (consumers decide 'about the life and health'). However, the increase in costs and prices of water resources, technologies, and wastewater treatment have raised the price of domestic water so that it has encouraged households to invest in saving equipment (Ward, White, 2014), not only for environmental or moral reasons, but purely for economic reasons – for example, the marginal cost of a dual-flush toilet will be returned in a few years. The problem for suppliers of domestic water is that consumption reduced in this way also means reduced sales for water treatment companies. Savings in operating costs as a consequence of reduced consumption will not result in savings of fixed costs, linked to long-term costly investments in infrastructure and supply networks (Cabrera et al., 2013). Efforts to cover the costs of reduced consumption will result in a further growth of the supply price. The response of consumers is becoming progressively smaller as price elasticity decreases. If the price of drinking water – for some consumer groups – affects their ability to afford enough water for direct consumption and sanitation (after the introduction of saving techniques in the household), the water and sewage tariff

within the household bills becomes independent and acts competitively against the affordability of other necessities of life. These processes can be described by the relationship between demand and household income (Parker, Wilby, 2013) and examination of the cross effects of necessities (Bakker et al., 2008). The issue of cost recovery for suppliers is associated with the problem of price affordability for consumers and the way in which it can act as a barrier to achieving the desired social and safety outcomes of domestic water supply (Hung, Chie, 2013). Paradoxically, the problem in question is not primarily a lack of water or neglected infrastructure, but the problem of economic management of supply and demand, which cannot be avoided even by developed countries. The ecological effects are impacted by this situation through knock-on pressures on the costs of collecting wastewater sewerage and treatment. For the operator of new publicly funded investment this represents a substantial increase in operating costs and, therefore, makes necessary an increase in sewage tariffs (François, 2010).

The points of demand dependence are derived from the equilibrium situation in each year, which is corresponded to by the price and the identified quantity of drinking water consumed. Both income and price elasticities of demand are defined as the proportion of annual increases in quantity and price (Haque, 2006), i.e.:

$$E_{DP} = \frac{Q_2 - Q_1}{(Q_1 + Q_2):2} : \frac{P_2 - P_1}{(P_1 + P_2):2}$$

$$E_{DI} = \frac{Q_2 - Q_1}{(Q_1 + Q_2):2} : \frac{I_2 - I_1}{(I_1 + I_2):2}$$

where:
E_{DP} = price elasticity of demand
E_{DI} = income elasticity of demand
Q_1, Q_2 = quantity (previous and current period)
P_1, P_2 = previous and current period prices
I_1, I_2 = previous and current period household incomes

The values are always expressed in positive numbers. Price elasticity estimates are generally found in the range of 0 to 0.5 in the short run and 0.5 to 1 in the long run. Income elasticity estimates are of a much smaller magnitude (usually) and positive. When calculating income elasticities of demand, statistical data describing aggregate domestic drinking water demand are used (in the Czech Republic). These data include approximately 94% of inhabitants which are supplied from public water systems and represent aggregate consumer behaviour. The remaining 6% include individual systems (water wells).

Further, water price elasticities are found to be higher in summer than in winter and price elasticities are generally the highest in situations where outside water usage is the highest (Griffin, Chang, 1991) including e.g. lawn and garden watering, car washing, and filling swimming pools. However, the research in the Czech Republic confirms no impacts of the season variables (temperature or rainfall) on drinking water consumption (Slavíková et al., 2013).

The dependence of the number of employees on the number of residents and the area of the town was calculated using the Pearson's correlation coefficient (r) (Hendl, 2012):

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

The correlation was selected as the preferred method because it expresses interdependence. The result was assessed using a test of statistical significance through setting a zero hypothesis.

RESULTS AND DISCUSSION

Three areas are worth discussing at this stage. Firstly, expected results correspond to the theory of consumer behaviour with increasing prices and/or with lower income effective consumer demand decreases. Secondly, parameters of income elasticity of demand for drinking water in different household groups (income deciles) to be expressed as short-term (average annual), medium-term (blocks of 4 years), and long-term (entire period, i.e. 8 years) response of demand with changing income. Thirdly, affordability of water and sewage tariff prices. The Czech Republic is included in OECD countries, where expenditure on domestic water, including sanitation services, exceeds the level of affordability proposed by Hung, Chie (2013) and meets the conditions for 'water poverty'technically defined as equal to or more than 2–3% of net household income.

The presented results are based on the use of household bills obtained from household budget survey. Calculations made concurrently using statistics concerning living conditions show somewhat different results. The differences between the calculations of weighted average price for water and sewage tariffs according to CZSO and Ministry of Agriculture data are small, and do not affect the overall results. The effect of changes in the VAT rate also did not show up markedly. A significant discrepancy can be found in the comparison of recalculated specific consumption of domestic water per person with the average statistically recorded and reported volume of billed water per supplied (connected) consumer. Additional and more detailed comparison carried out on the basis of household incomes and living conditions confirmed the overall picture and the tendency to social stratification in domestic water consumption in the Czech Republic.

Table 1. Development of drinking water price and quantity of domestic consumption in the Czech Republic

Year	1994	1995	1996	1997	1998	1999	2000	2001
Current price (CZK)	9.39	10.70	12.08	13.64	15.25	16.73	18.00	19.06
Domestic consumption (mil. m^3)	696	656	631	604	580	564	554	536

2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
20.47	21.56	22.76	23.58	24.56	25.83	28.56	30.63	32.01	33.88	37.28
545	547	543	531	528	532	517	505	493	486	481

basic data: CZSO, MoA, MWRI

Behaviour of consumers

The behaviour of consumers of domestic water in the Czech Republic corresponds to theoretical assumptions (O E C D , 2011). It confirms the so far prevailing influence of economic drivers, prices, and financial constraints for households, on this situation (compared to social, health, etc.). The results are not sufficient as evidence for the definition of demand as part of the water market, but they confirm the effectiveness of the application of general economic methods to identify the characteristics of demand. The demand curve can be constructed using empirical data of price development, using current prices, or using prices net of inflation and value added tax. Connection by the best relation to reliability gives smooth curves (Fig. 1, original source of data is mentioned in Table 1) corresponding to the theoretical course of demand. To assess the level of dependence, Pearson's correlation coefficient was used. Its value $r = 0.91965$ expresses the high level of dependence, which reflects a decline in domestic consumption with the rising price of drinking water. In testing statistical significance there was assessed the zero hypothesis with the assumption that a decline in domestic consumption is not dependent on the price of drinking water. The test rejected this hypothesis and confirmed the dependence.

Income elasticity of demand

The demand for drinking water in most of the monitored situations in households (70 points, characterized by the parameter of income elasticity) shows two different types of behaviour depending on the period: rather inelastic demand (< 1) and rather elastic demand (> 1) (Fig. 1). Short-term (annual) elasticity (AverYears) is on average higher than medium-term (Med 2Term) and long-term elasticity (LongTerm). Medium-term elasticity in the second 4-year period is higher (see Fig. 2). While the long-term elasticity confirms the character of domestic water as the necessity (less dependent on conditions), the evident difference in income deciles should be interpreted with caution as a more significant link between demand and income is to be expected in lower income groups. The increasing sensitivity of consumption relative to income in the second (later) 4-year period can be considered as an indicator of the growing importance of water services within the framework of housing. It is also interesting to

Fig. 1. Drinking water demand in the Czech Republic
basic data: CZSO, MoA, MWRI

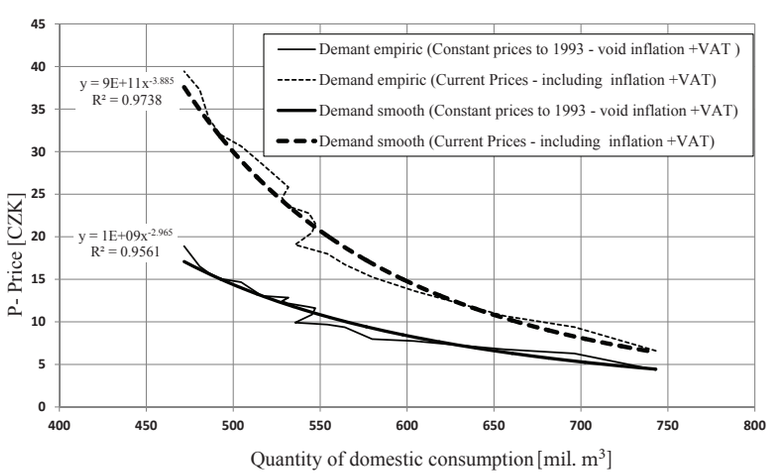

Table 2. Domestic water and sewage bills as a percentage of the net budget of households

Year	Income deciles (%)									
	1	2	3	4	5	6	7	8	9	10
2005	**2.72**	1.83	1.74	1.58	1.51	1.42	1.21	1.16	1.07	0.80
2006	**2.70**	1.87	1.66	1.61	1.44	1.46	1.29	1.22	1.12	0.74
2007	**2.71**	1.83	1.62	1.53	1.50	1.46	1.25	1.16	1.03	0.73
2008	**2.52**	1.73	1.57	1.57	1.49	1.39	1.30	1.18	1.06	0.80
2009	**2.52**	1.67	1.59	1.52	1.50	1.43	1.32	1.19	1.12	0.76
2010	**2.57**	1.78	1.70	1.57	1.57	1.44	1.35	1.22	1.10	0.80
2011	**2.72**	1.88	1.79	1.67	1.66	1.43	1.44	1.25	1.19	0.86
2012	**2.74**	**2.06**	1.76	1.88	1.72	1.63	1.49	1.44	1.20	0.91

water tariff: prohibitive vulnerable affordable

basic data: CZSO, MoA, MWRI

compare two medium-term periods, of which the later falls in with the conditions of economic crisis.

Affordability

Affordability of drinking water in the Czech Republic (P e t r u ž e l a et al., 2009) is indicated by stratification by income and the development over time (Table 2). The results derived from water bills suggest that the problem of water poverty – in the definition mentioned above (R e y n a u d , 2007) – covertly occurs in about a third of households throughout the monitored period. It is evident that the number of households falling into this group increases to reach as much as 25% in the years affected by the decline of economic growth. If we use water services expenditure > 2% in the net household income as the critical indicator for water poverty, then the data shows an affordability gap growing between the two parts of the population: households in income decile 4 and below, compared with households in income decile 5 and above. For income decile 4 the latter years of the time series (2009 onwards) represent a shift to water poverty which is concurrent with recession and economic measures introduced to improve economic performance.

CONCLUSION

Based on the results of the investigation it is clear that a current, and very important, issue in sustainable water supply management and sanitation enterprises is decreasing consumption, which has a negative economic and social impact. This is also associated with the

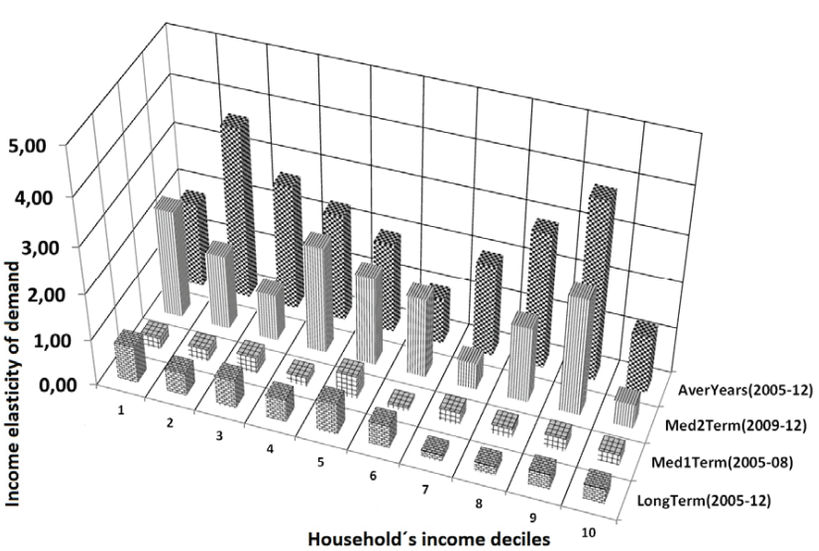

Fig. 2. Agregate demand income elasticity in the Czech Republic
AverYears = short-term (annual) elasticity, MedTerm = medium-term elasticity, LongTerm = long-term elasticity
LongTerm data express the difference between the values reached in the first and the last year of the reference period; AverYears data express the average for individual years of the reference period
basic data: CZSO, MoA, MWRI

method of price regulation by the government, since it affects domestic water consumption and therefore supports these negative tendencies.

The investigation was based on the structured statistical series from the Household Budget Survey (including water services payments) and parallel statistics on household consumption of domestic water, plus water and sewage tariffs in the Czech Republic in 2005–2012. This confirmed the applicability and effectiveness of economic methods, derived from the theory of consumer behaviour, by investigating the behaviour of drinking water demand. It allowed the identification of the social impact on domestic water consumption, which indicates, as expected, a negative correlation between price and volume of consumption and a positive correlation between consumption and increasing household income. The long-term income elasticity ranges are mainly in the zone of inelastic demand, less than 1, and so consumption responds to changes in income rather slowly. The response is higher in the first, compared to the second, monitored 4-year period. Further research may confirm, *inter alia*, the effectiveness of social modification of the price tool – water tariff in the form of block rates (O E C D , 2011) or warn against optimism in recovery of drinking water demand by using pricing manipulations.

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ECONOMIC EFFICIENCY OF SELECTED CROPS CULTIVATED UNDER DIFFERENT TECHNOLOGY OF SOIL TILLAGE*

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The objective of this study was the model comparison and economic evaluation of different methods of soil tillage and crop stand establishments used. Based on yield results (winter wheat, spring barley, and white mustard cultivated in three-crop rotation) from field experiments with conventional, conservation with minimum tillage, and no-tillage methods conducted at the site Prague-Ruzyně, model economic balances were evaluated. Prices of the main products were determined based on the yield results from the period 2010–2013 and the current market prices. In the individual tillage systems, the total costs of production of evaluated crops were counted up and profitability was calculated as a ratio of profit to total costs. The highest total costs of crop cultivation were identified in cereals under conventional soil tillage, on the contrary, the lowest in cereals cultivated under conservation tillage technology. As for the growing technologies, the highest profitability was found in winter wheat, as for the tillage methods, it was in the conservation variant with minimum tillage. The economic evaluation for individual crops was based on standards of growing technologies and particular work operations.

winter wheat; spring barley; white mustard; different soil tillage intensity; production; economic balances

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INTRODUCTION

When farmers use different soil tillage technologies and methods of crop stand establishment for a long time, then they are more interested in consequences of particular cultivation measures on the soil environment. In addition to conventional technology based on ploughing, minimization methods of soil tillage without ploughing are becoming increasingly important. Topsoil is loosened into smaller depth without turning over, or conservation measures are applied where after crop seeding at least 30% of soil surface remains covered with post-harvest residues or catch-crop biomass.

Utilization of organic matter supports higher microbial activity in soil improving most of soil fertility parameters (Dzenia et al., 1999; Šimon, Javůrek, 1999; and others). Direct drilling into no-tilled soil using special sowing machines is the extreme variant of minimization technologies of crop stand establishment without ploughing.

Apart from soil properties of the given site, weed status of land, appropriate machinery in the agrocompanies, also economic efficiency of crop production is one of the key issues deciding about tillage technology used in the final stage.

Results to date from the field experiments and experience from agricultural practice demonstrate the favourable influence of minimization tillage technologies on economy of crop production through work operation decrease and subsequent decrease of direct costs, lower fuel consumption, and labour needed per production unit (Kovacev et al., 2011).

A permanent rise in fuel as well as labour prices increases the differences in costs among conventional technologies using a various rate of minimization elements (Hůla et al., 2008).

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Minimization technologies of cereal production are labour-saving which reduces the total costs of growing technologies. Also the costs of fuel and use of machines, which have a smaller number of passages, are lower. However, in minimization technologies we have to take into account higher expenditures on weed control (more expensive herbicides) which are obviously reflected in the total costs (H o r á k , 2005).

For this reason, we focused on the evaluation of long-term effect of different soil tillage technologies on the total production and economic efficiency of winter wheat, spring barley, and white mustard cultivation.

Based on yield results from the field experiments, model economic balances were evaluated for particular soil-climatic site conditions and given agronomic measures. The objective of this study was the model comparison and economic evaluation of different methods of soil tillage and crop stand establishments used.

MATERIAL AND METHODS

The data for the evaluation of economic balances of cultivated crops, i.e. winter wheat, spring barley, and white mustard in three-crop rotation, were derived from a plot field experiment established at the site Prague-Ruzyně in 1995. During the years 2010–2013 three different methods of soil tillage and crop stand establishment were applied, and subsequently total yields were evaluated and all work operations, material consumed, and concrete use of farm machines were recorded by each of the individual technologies used.

The experimental plots (plot size 24 m^2) are located in a temperate semiarid climate, 350 m a.s.l., with an annual mean air temperature of 7.9 °C, and mean annual precipitation of 477 mm. The field site has a soil of clay-loam texture (Orthic Luvisol, FAO Taxonomy). As an experiment design a split-plot method with four replications was used. The P and K fertilization was uniform (54 kg P_2O_5 per ha in superphosphate and 100 kg K_2O per ha in potassium salt yearly). For economic evaluation the annual divided doses of nitrogen in the total dosage of 100 kg for wheat, 80 kg for barley, and 30 kg (single dose) for mustard per ha were included. For calculation, the average total yields from the field experiments for four years (2010–2013) were used.

Cultivation practices evaluated

Conventional soil tillage and crop stand establishment (CT) included post-harvest stubble breaking, mouldboard ploughing to a depth of 0.20 m, usual seed-bed preparation, sowing, rolling, N-fertilization, harrowing, treatments of crops against weeds and pests, harvest and grain transport, crushing of straw by a harvester adapter.

Conservation soil tillage and crop stand establishment (MT) included shallow disking (about 10 cm deep) with crushed straw incorporating, seed-bed preparation and levelling of soil surface with vibratory or rotary harrows, sowing, N-fertilization, pesticide treatments, harvest and grain transport, crushing of straw by a harvester adapter.

Crop stand establishment by no-tillage practice (NT) included direct drilling into no-tilled soil by special drill machine, N-fertilization, pesticide treatments, harvest and grain transport, baling of straw and its transport to storage.

To ensure comparable crop stand structure in all tillage variants, all crops (including conventional tillage treatment) were sown using a John Deere 750A drill machine (John Deere International GmbH, Schaffhausen, Switzerland).

The economic evaluation for the individual crops cultivated under different soil tillage systems was based on the standards of cultivation methods and the particular work operations for monitored crops according to current regulations (www.agronormativy.cz). The same source was used to determine prices of seeds, fertilizers, and agrochemicals consumed and the average farm prices of crop products.

For cultivation technologies of individual crops and finally for the whole crop rotation, the total costs (variable + fixed of machinery) converted to 1 ha were calculated. Variable costs included material costs and mechanized operations, fixed costs comprised rent for land, taxes, depreciation and repair of buildings, machinery depreciations, interests, production and administrative overheads. According to yields achieved in the field trials in the period 2010–2013 and actual market prices, the prices of the main products were determined. Furthermore, profitability of the individual crops under different soil tillage methods was calculated as a rate of profit and/or loss to the total costs. The mentioned prices do not include VAT.

RESULTS

Production

In the particular years assessed, relatively significant differences in the yields of the main products (grains/seeds) of the crops monitored were recorded (Table 1). In winter wheat the yields varied on average from 6.65 t ha^{-1} in 2012 to 10.05 t ha^{-1} in 2013; similarly in spring barley from 6.61 t ha^{-1} in 2012 to 9.25 t ha^{-1} in 2013. The yields of mustard seed were comparable within the years.

In 2012, when grain production of cereals was the lowest in the given period, the above-average temperatures and below-average rainfalls were recorded in the periods critical for yield formation. For example, during the period from April to September the temperature at the Ruzyně site was on average 1.8 °C

Table 1. Average seed /grain yields (t ha^{-1}) of crops tested under different soil tillage methods in the selected period

Crop	Method	2010	2011	2012	2013	Average
Winter wheat	CT	9.46	9.15	6.23	9.68	8.63
	MT	10.57	10.61	6.79	10.08	9.51
	NT	9.27	9.42	6.93	10.40	9.00
	Average	9.77	9.73	6.65	10.05	9.05
Spring barley	CT	8.50	7.54	6.09	9.21	7.84
	MT	8.43	8.71	6.87	9.25	8.31
	NT	8.40	8.59	6.86	9.29	8.28
	Average	8.44	8.28	6.61	9.25	8.14
White mustard	CT	1.82	1.69	1.88	1.87	1.81
	MT	1.79	1.91	1.95	1.85	1.87
	NT	1.93	1.76	2.03	1.80	1.88
	Average	1.85	1.79	1.95	1.84	1.86
Sum of average grain yields in crop rotation	CT	19.78	18.38	14.20	20.76	18.28
	MT	20.79	21.23	15.61	21.18	19.69
	NT	19.60	19.77	15.82	21.49	19.16

Table 2. Economic evaluation of conventional soil tillage treatment (CT) in CZK ha^{-1}

Crop	Price of material consumed	Costs variable+fixed of machinery	Total without VAT	Fuel consumption (l ha^{-1})	Hum. labour need (h ha^{-1})
Winter wheat	10 549	7 861	18 410	75.40	5.42
Spring barley	11 565	7 764	19 329	75.90	5.81
White mustard	7 484	7 029	14 513	69.05	4.72
Total	29 598	22 654	52 252	220.35	15.95

Table 3. Economic evaluation of conservation (reduced) soil tillage treatment (MT) in CZK ha^{-1}

Crop	Price of material consumed	Costs variable+fixed of machinery	Total without VAT	Fuel consumption (l ha^{-1})	Hum. labour need (h ha^{-1})
Winter wheat	10 879	5 634	16 513	46.50	3.82
Spring barley	10 579	6 101	16 680	52.60	4.47
White mustard	7 704	5 948	13 652	50.35	4.17
Total	29 162	17 683	46 845	149.45	12.46

Table 4. Economic evaluation of no - tillage treatment (NT) in CZK ha^{-1}

Crop	Price of material consumed	Costs variable+fixed of machinery	Total without VAT	Fuel consumption (l ha^{-1})	Hum. labour need (h ha^{-1})
Winter wheat	11 224	6 842	18 066	55.10	5.07
Spring barley	11 604	6 779	18 383	54.60	4.97
White mustard	8 159	6 621	14 780	56.65	5.07
Total	30 987	20 242	51 229	166.35	15.11

Table 5. Grain/seed yields (t ha^{-1}), total costs of production (CZK ha^{-1}) and profitability (%) of the individual crops and entire crop rotation with respect to different soil tillage method

Crop	Technology	Average yield (t ha^{-1})	Total main product price (CZK ha^{-1})	Total costs of production (CZK ha^{-1})	Profitability (%)
Winter wheat	CT	8.63	43 582	18 410	136.7
	MT	9.51	48 026	16 513	190.8
	NT	9.00	45 450	18 066	151.6
	Average	9.05	45 686	17 663	159.7
Spring barley	CT	7.84	40 352	19 329	108.8
	MT	8.31	42 772	16 680	156.4
	NT	8.28	42 617	18 383	131.8
	Average	8.14	41 914	18 131	132.3
White mustard	CT	1.81	27 389	14 513	88.7
	MT	1.87	28 297	13 652	107.3
	NT	1.88	28 448	14 780	92.5
	Average	1.85	28 045	14 315	96.2

Notes: CT = conventional tillage; MT = minimum tillage; NT = no-tillage

above the long-term average. The total sum of rainfall was influenced by the record amount of precipitation (138 mm) in the first decade of July, however in other months the precipitation was significantly lower if compared to long-term average. Whereas in 2013, when grain yields of cereals were the highest, the course of temperature and rainfall during the vegetation period was favourable, except an excessive sum of precipitation (370% of normal) in June.

Average yields of individual crops monitored and the whole crop rotation in the period 2010–2013 under different soil tillage methods and crop stand establishment are shown in Table 1. Four-year average results show the highest yields of winter wheat by the conservation soil tillage variant with minimum soil cultivation (9.51 t ha^{-1}), the lowest wheat grain production (8.63 t ha^{-1}) by the conventional tillage treatment. The differences of grain production represent 3.8%.

As for spring barley, the highest grain production (8.31 t ha^{-1}) was recorded by the conservation variant with minimum tillage as well, in other tillage treatments, the yields were slightly lower.

The seed production of mustard was comparable in all years of the experimental series. On the plots with conservation tillage treatment, higher seed yields (1.88 t ha^{-1}) than in the other variants (1.81 and/or 1.87 t ha^{-1}) were registered. In the frame of the whole crop rotation, yields of the main product on plots with different tillage technology were considerably stable during the given period; higher production was recorded in variants of reduced tillage method (19.69 t ha^{-1}), lower one in conventional tillage treatment (18.28 t ha^{-1}), which represents a difference of 7.2% (Table 1).

Economics

A model evaluation of production of all crops and the whole crop rotation under different soil tillage management from an economic viewpoint was carried out. Based on four-year results and work operation used, variable costs were determined and then calculated (Tables 2–4).

The highest fuel consumption was found in all crops cultivated under conventional tillage method. On the other hand, the lowest fuel consumption was recorded at conservation tillage. For the entire crop rotation, it was 220.35 l ha^{-1} in conventional tillage technology and 149.46 l ha^{-1} in conservation tillage method which represents saving of about 70 l ha^{-1}. Similarly, the need of human labour was the highest under conventional crop cultivating, particularly in cereals and for the whole crop rotation as well, the lowest in conservation tillage method. This is due to the necessary use of more labour-intensive operations in conventional technology, especially by the costs of medium deep ploughing. Conservation method based on minimum tillage required more operations, especially in plant protection, than the other tillage systems evaluated. The highest total costs were found in cereals under conventional tillage method, the lowest also in cereals, but under conservation minimum tillage system. In winter wheat, the total costs were 16 513–18 410 CZK ha^{-1} (Tables 2, 3), in spring barley 16 680–19 329 CZK ha^{-1}.

For all crops in crop rotation cultivated under conventional tillage method, the total costs were 52 252 CZK ha^{-1}, in conservation (minimum) soil

tillage variant 46 845 CZK ha^{-1}, and under no-tillage treatment 51 229 CZK ha^{-1}, which represents a difference of 6 407 (10.3%) and/or 1 023 (2.0%) CZK ha^{-1} compared with conventional soil tillage treatment (Tables 2–4).

When comparing the individual crops, the highest profitability was found on average in winter wheat (159.7%), then in spring barley (132.3%), and the lowest in white mustard (96.2%) – see Table 5. As for soil tillage methods, regardless of crops, the highest profitability was attained in the conservation variant with minimum tillage and the lowest (except spring barley) in conventional tillage treatment (Table 5).

Total costs in Table 5 are calculated without VAT, straw production was not included in this evaluation, and revenue does not include any subsidies. A farmer may apply for subsidy; in the year 2012, the subsidies represented 5 387.30 CZK ha^{-1} for SAPS (Single Area Payment Scheme) and 491 CZK ha^{-1} for TOP-UP (National Additional Payments to direct aids)**.** In standards for economic evaluation (www.agronormativy.cz), a wide-range subsidy in the amount of 6 068.88 CZK ha^{-1} is cited.

DISCUSSION

The obtained yield results are obviously higher than those presented as an average in agricultural practice. For example, according to the Section of Agricultural Commodities of the Ministry of Agriculture of the Czech Republic (MZE Report 2013a, b), average grain yield results during the period 2009–2013 were 5.20 t ha^{-1} in winter wheat, 4.44 t ha^{-1} in spring barley, and 0.85 t ha^{-1} in white mustard. Also the information source www.farmprofit.cz for the cost calculation by production area gives the following results: yield of winter wheat 4.69–4.98 t ha^{-1}, yield of spring barley 4.39–4.81 t ha^{-1}. The farm Agro Žlunice, located in favourable soil and climatic conditions (sugar beet production type area), in winter wheat achieved an average grain yield of 7.10 t ha^{-1} in the period 2010–2012 (Homolka, Bubeníková, 2013). The above mentioned data are average regardless of the tillage technology used.

Kovacev et al. (2013) mentioned the great influence of weather conditions on production of spring barley and the occurrence of dry periods during the vegetation season, which could have negative effect on crop yields. The highest average yields in barley production near Staro Petrovo Selo (Croatia) oscillated around 3.20 t ha^{-1} (Kovacev et al., 2013).

Dzenia et al. (1999), Reinhard et al. (2001) and many other authors found minimum and insignificant yield difference between soil tillage methods of different intensity. Šimon, Javůrek (1999) presented the results from exact field experiments on fertile chernozem, where yields of cereals were significantly higher in conventional variant than after drilling into no-tilled soil. From this short review it is evident that the results of the study of the soil tillage impact on crop yields are different and their dissimilarity logically stems from different soil and climatic conditions of the sites.

Hůla et al. (2008) mentioned yield results of winter wheat from the field trials in the maize production type area (Hrušovany u Brna) under different soil tillage technologies. The average highest grain yield (6.62 t ha^{-1}) was achieved after the shallow tillage method, then after direct sowing of winter wheat into no-tilled soil (6.58 t ha$^{-1)}$, and finally the lowest yield (6.49 t ha^{-1}) was recorded after mouldboard ploughing. In the sugar beet production type area (Ivanovice na Hané), the highest yield (6.47 t ha^{-1}) was recorded after the shallow tillage technology as well. After ploughing and direct sowing into no-tilled soil the average yields of winter wheat were slightly lower (6.44 t ha^{-1} in both).

Similarly, the long-term yield results of spring barley from the sugar beet production area (the 15-year average) confirmed that shallow soil tillage is fully sufficient for achievement of high grain production (Hrubý et al., 2007). The average grain yield achieved under shallow ploughing (up to 0.15 m) was 6.55 t ha^{-1}, under medium deep ploughing (up to 0.22 m) it was 6.38 t ha^{-1}, and under sowing into no-tilled soil the yield was 6.43 t ha^{-1}.

It can be stated that the grain production of experimental cereals in particular evaluated variants of soil tillage in the present study is comparable with the conclusions given in literature (Hrubý et al., 2007; Hůla et al., 2008).

And now to economics. Kovacev et al. (2011) studied economic efficiency of non-conventional tillage systems in winter barley production. A comparison showed that conventional system had the highest fuel consumption (50.93 l ha^{-1}). The most economical system in crop production (33.03 l ha^{-1} with a decrease of 35.1%) was identified in RT1 variant (chisel plough, disc harrow seed-bed implement, drill).

Kavka et al. (2006) reported total technological costs according to cultivation intensity for food winter wheat 15 509–23 618 CZK ha^{-1}, for malting spring barley 15 934–22 302 CZK ha^{-1}, and for white mustard 9 668–16 030 CZK ha^{-1}. Total costs according to cultivation intensity published in standards (www.agronormativy.cz) are 19 793–29 296 CZK ha^{-1} for food winter wheat, 20 760–27 407 CZK ha^{-1} for malting spring barley, and 16 902–21 631 CZK ha^{-1} for white mustard.

According to the website www.farmprofit.cz, in 2012 the total actual costs on average of the production areas were 22 677 CZK ha^{-1} for winter wheat and 20 781 CZK ha^{-1} for spring barley. Similarly, the Research Institute of Agricultural Engineering, Prague-Ruzyně in the normatives for counselling

gave the model total costs of growing operations according to production areas for winter wheat 20 683–21 897 CZK ha^{-1}, for spring barley 18 772–19 066 CZK ha^{-1} (www.vuzt.cz). Homolka, Bubeníková (2013) mentioned the total direct costs established for a concrete example in practice for winter wheat, depending on the year, from 16 345 to 19 437 CZK ha^{-1}. However, all the above mentioned data do not distinguish the differences of the individual soil tillage methods.

Nave et al. (2013) found out that the medium-input variant is the most efficient with the best economic results for any wheat price. On the other hand, the high-input type has a lower economic performance.

As for the particular technologies of soil tillage for cereals growing, we may say that with decreasing soil tillage intensity the costs for growing technology decrease, too. But in no-tillage technology the costs may rise owing to the necessity of non-selective and more effective (more expensive as well) pesticides application and higher nitrogen doses to reach a comparable yield with conventional technology. But shallow tillage with crushed straw incorporated has proved to be cheaper.

CONCLUSION

The highest average grain production of winter wheat was achieved in the experimental variant of conservation land management (MT) with minimum tillage, the lowest one under conventional tillage method (CT). The same results were recorded in spring barley.

The highest average seed production of white mustard was recorded in the no-tillage variant (NT), in other tillage variants the yields of seeds were insignificantly lower.

The highest total costs of cultivation technology were found in cereals under CT method, the lowest ones in cereals as well, but cultivated using MT variant with minimum tillage.

Production costs of crops cultivated must be calculated case by case, because it depends on many factors – soil-climatic and other site conditions, method of stand establishment and crop harvesting, number of necessary work operations (fertilization, pest control and others), transport distances, storage method etc., all depending on the level of yield achieved by the cultivated commodities.

The highest profitability of cultivating methods was identified in winter wheat, then in spring barley, and the lowest in white mustard. Regarding the tillage methods, the highest profitability values were found in the MT variant, the lowest ones (except spring barley) under CT technology.

Regarding the choice of tillage systems, assuming the relative uniform level of yields, priority should be given to systems with lower tillage intensity level, which are cost-saving and simpler to manage due to less need of machines and human labour.

Reduced soil tillage treatment with straw and post-harvest residue incorporation (conservation soil tillage) was proved to be cheaper than the other two tillage methods.

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