The results of manipulated experiments with inoculation of *Ips typographus* (L., 1758) to spruce trees under various levels of water stress

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ABSTRACT: Manipulated experiments with males of *Ips typographus* (L., 1758) were conducted in spruce stands in north-western Slovakia. Some of trees were stressed by a lack of water caused by preparation of roofs under canopy. Inoculation experiments with bark beetles were conducted on such trees. According to results, the differences in attack rates between differently positioned trees on slope were not statistically significant (P = 0.389 for bottom and middle and P = 0.924 for bottom and top, and P = 0.530 for middle and top trees, *t*-test). Also the differences in attacks rate and the speed of entry holes preparation between more stressed and less-stressed trees were not statistically significant (P = 0.321, *t*-test). Thus the results of inoculation confirmed that low level of water stress does not lead necessarily to higher attack rate and (neither) faster speed of entry holes preparation. The obtained results are discussed.

Keywords: *Ips typographus*; spruce; water stress; manipulated inoculation experiments

Natural tree composition has been dramatically changed across Europe. Today, distributional ranges of several tree species are mainly determined by former management practices rather than by natural factors (Ellenberg 1986). As a result of social and economy development, there appeared vast regions of non-native monocultures, suffering from problems in forest health. In many of these regions, the forest decline has recently been subject of concern (Blank et al. 1988; Führer 1990; Kandler, Innes 1995). Large area of such stands along with compound of biotic and abiotic stressors (air pollution, degradation and compaction of soil, nutrients exhaustion, increased activity of pests and pathogens) resulted to permanent forest health problems. This is particularly true for secondary Norway spruce (*Picea abies* [L.] Karst.) stands in Central Europe that have been already weakened due to the impact of extreme climate conditions within the past 20 years and secondary pest and disease infestation (Jankovský, Cudlín 2002; Holuša, Liška 2002). However, no statistical relation was found between forest decline symptoms and bark beetle attacks in study of Prien et al. (1996).

Several studies have been performed in order to assess the factors affecting the susceptibility of stands to bark beetles attack. Multiple regression analyses indicate that altitude and soil nutrients, such as nitrogen, phosphorus, and magnesium, have a significant influence on *Ips typographus* (L., 1758) attacks rate (Nef 1994; Dutilleul et al. 2000). Under favourable conditions, *I. typographus* is able to attack healthy trees and it is a primary factor causing direct tree mortality (Christiansen, Huse 1980; Christiansen 1989). Outbreaks can develop rapidly in spruce stands that are damaged by wind (Capecki 1978; Lindelöw, Schroeder 2001), snow (Schroeder, Eidmann 1993), or air pollution (Baltensweiler 1985; Christiansen 1989). Windstorms are especially important pre-

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cursors to outbreaks because they quickly provide large quantities of breeding material in the form of broken or fallen stems (Capecki 1981; Göthlin et al. 2000; Mihalciuc et al. 2001). Many evidences exist about the high susceptibility of the trees to bark beetles (mainly *I. typographus* L.) after exposure to the sun subsequently after opening the canopy (Lobinger, Skatulla 1996; Jakuš 1998). There are also indications that trees stressed by drought (Grodzki 1998; Grodzki et al. 2002) are more infested by bark beetles, but quantitative study of this relation is not easy.

Thus, the main goal of this paper is to analyze the behaviour of bark beetles on trees under various regime of water stress in declining spruce monocultures. Within the frame of which we focus on:
– analysis of *I. typographus* attacks rate on trees with various position on slope and on stressed versus unstressed trees;
– analysis of *I. typographus* attacks rate and speed of boring on more stressed versus less stressed trees.

METHODOLOGY

The effect of water stress on the attack of *I. typographus* males has been studied by two experimental designs:
– on the trees stressed by their various position on slope (bottom, middle part, top of slope),
– on the trees stressed versus not stressed by elimination of precipitation.

Preparation of *I. typographus* males to experiments

Acquisition of wild individuals. To collect adults of *I. typographus*, 5 pheromone traps were installed in central Slovakia in spring, which were baited by pheromone dispensers. In the peak of spring flight, the traps were cleaned each hour and non-damaged individuals were collected. These individuals were put into refrigerator into wet environment and later transported to lab. After several days, the number of individuals was enough high to establish artificial rearing.

Laboratory rearing of I. typographus. The rearing cages were used for breeding of beetles on spruce bolts. Bolts were prepared from non-infested spruce logs 1 month prior breeding started. Selected non-damaged individuals were tested on motion and than released to rearing cages. They mated and established new generation. The breeding was conducted in natural temperature and light conditions to have adults at the end of June. Bolts were watered and treated by anti-fungal solution if necessary. Emerged individuals were taken away each day.

Storing the emerged individuals. The emergence of individuals usually takes longer time. The manipulated experiments requested several hundred individuals and thus fresh adults were stored in refrigerator in specific conditions where they were able to survive several days.

Sexing the adults for experiments. After establishment of water stress experiments, the stored adults were sexed, because only males were tested in manipulated experiments. Sexing was done according a paper of Schlyter and Cederholm (1981). According to this paper, males have bigger frontal projection and less dense hairs cover on front. Sexed males were stored again in refrigerator. Prior experiments, they were transported in the field refrigerator, released for 1–2 hours for adaptation and than used into experiments.

Establishment of experiments. Selected males were released to small ampoules (Fig. 1), which

Fig. 1. One-way choice experiment established on stressed tree at August 18th 2006 (Kysuce – Šadibolovci)

were fixed to trees without possibility for males to escape = 1-way choice experimental design. Bored dust was collected and the frequency of attack and the depth of entry holes were measured. Simultaneously, the water regime of each tree was recorded by sap-flow meter. Ten males were inoculated at each of 15 trees. Prior maternal chamber was started to be prepared, the beetles were removed from the trees and damage was treated by resin.

"One way choice" experiments were conducted in 3 various days when parameters of water stress were predicted to be as different (July 19th and 27th, August 18th 2006). Prior these experiments, the preliminary tests of all parameters were done at the beginning of July (e.g. to eliminate complete formation of entry hole and start attractant production).

The real experiments were conducted on 2 different sites situated 1 km from each other. Site 1 consisted of 3A, B, C triples of trees (bottom = n. 3, middle = n. 4 and top = n. 5A, B, C of slope) where water regime was evaluated. Site 2 consisted of 2 triples of trees. Each tree in the 1st triple was manipulated by plastic roofs, which prevented precipitation to enter soil under roof (labeled in Tables 1 and 2 as S = with roof), another one was without roof (B = without roof). The results of frequency and the depth of entry holes (stressed versus unstressed) were tested by sign test and *t*-test respectively. The results of this experiment were subsequently compared to data which were obtained by sap-flow meter. Because the experimental design of sap-flow meters, the data obtained were not continuous and we were only able to evaluate qualitative parameters of water stress (we were able to evaluate that lower stress was on non-manipulated trees than on trees with roofs).

RESULTS AND DISCUSSION

Preliminary experiments have been conducted at the beginning of July 2007 at research area Šadibolovci. It was necessary to evaluate the speed of entry hole preparation (Fig. 1), to prevent establishment of mating chamber, attractant production and infestation of experimental tree by additional individuals. Experimental trees were used by several research groups and it was highly needed that trees remained on the place whole season. Briefly: 10 males were released to each of experimental trees and the speed of boring was estimated each 2 hours. One tree was in shadow and one was on direct sunlight. Males started with boring 2 hours after inoculation and 6 hours later were fully bored (but they did not start to prepare mating chamber). Thus, the maximum span of subsequent inoculation experiments was stated to be 6–8 hours.

Bottom triple (trees 3), was attacked by lower number of beetles as medium (trees 4) and top (trees 5) triples (Table 1). The sign test suggested that these differences were statistically not sig-

Table 1. Number of entry holes produced by males into individual trees

Tree	1st checking	2nd checking	Tree	1st checking	2nd checking	Tree	1st checking	2nd checking
July 19th 2006								
5A	0	0	4A	1	3	3A	0	0
5B	1	3	4B	1	1	3B	1	2
5C	1	3	4C	0	2	3C	0	1
Total	2	6	Total	2	6	Total	1	3
July 27th 2006								
1S	0	1	1B	2	2			
2S	0	2	2B	0	2			
3S	4	5	3B	1	3			
Total	4	8	Total	3	7			
August 18th 2006								
1S	2	2	1B	0	1			
2S	0	0	2B	2	4			
3S	1	1	3B	2	4			
Total	3	3	Total	4	9			

nificant (P = 0.577 for 3–5 and 4–5, P = 0.000 for 4–5 in case 1st checking; P = 1.000 for 3–5 and 4–5, P = 0.000 for 4–5 in case 2nd checking). Table 2 presents average depth of entry holes calculated from 10 individuals on each tree in mm. The 1st checking was done after 2 hours, the 2nd one after additional 4–5 hours, when the most active males were able completely bored into the tree.

Statistical significance of differences in average depth of entry holes (Table 2) was not confirmed on the base of data from the 1st checking. The differences between trees 3 and 4 were not significant (P = 0.573, t-test). The results were same between 3 and 5 (P = 0.573, t-test) and between 4 and 5 (P = 1.000, t-test). Test showed similar results also after the 2nd checking (6 hours later – P = 0.389 for 3 and 4 and P = 0.924 for 3 and 5, and P = 0.530 for 4 and 5).

The end of July 2006 was characterized by relative lack of precipitation but differences between the number of individuals bored into stressed and control trees were not statistically significant (P = 0.378, sign test for 1st checking; P = 0.258, sign test for 2nd checking). The experiments were time-consuming and thus, only 10 individuals were inoculated to each tree which represents a low attack rate. It would be necessary to inoculate higher number of individuals to simulate mass attack in the future. Experiments above were conducted on the base of trees and this is not optimal place for attack. Additional experiments might be conducted on place of the most frequent attack – below the beginning of the green canopy.

Also average depth of entry holes in this experiment was not significantly different for the 1st (P = 0.423, t-test) and also for the 2nd (P = 0.321, t-test) checking.

After higher amount of precipitation and improvement of water regime in August 2006, the frequency of attacks increased on controlled trees which were not stressed by manipulated drought. We expected opposite results, but similar situations are also known in literature when Reeve et al. (1995) suggested that lower level of water stress mobilize the defence mechanism of stressed trees what subsequently leads to an increase of resin production. Simulation of such attack may lead to a decrease of frequency number of entry holes in manipulated experiments on stressed trees.

The same situation was observed on the same trees also in experiments in August 18th. Statistical significance of differences was not confirmed either for the 1st (P = 0.557, t-test), nor for the 2nd checking (P = 0.291, t-test).

Experiments were conducted in spruce stands which are characterized by radial increment almost equal to 0, which suggest that assimilatory apparatus of trees have not worked well for the whole experimental time. Partial explanation of such phenomena gives measurement of water regime on the same trees, which suggested some level of water stress in July 2006 (higher) and in August (lower).

Table 2. Average depth of entry holes on experimental trees

Tree	1st checking	2nd checking	Tree	1st checking	2nd checking	Tree	1st checking	2nd checking
July 19th 2006								
5A	0	0	4A	0.1	0.8	3A	0	0
5B	0.1	0.3	4B	0.1	0.7	3B	0.1	1.2
5C	0.1	0.9	4C	0	0.7	3C	0	0.1
Total	0.067	0.4	Total	0.067	0.733	Total	0.033	0.433
July 27th 2006								
1S	0	0.1	1B	0.1	0.5			
2S	0	0.2	2B	0	0.9			
3S	0.4	0.7	3B	0.1	0.6			
Total	0.133	0.333	Total	0.067	0.667			
August 18th 2006								
1S	0.2	0.5	1B	0	0.4			
2S	0	0	2B	0.2	1			
3S	0.05	1	3B	0.2	1.4			
Total	0.083	0.5	Total	0.133	0.933			

The amount of precipitation in July was low and the effect of this fact we observed via continual drying the trees in that time (with exception of several days in the mid of July when precipitation was observed). The beginning of August was quite different when trees have not suffered by water stress. Subsequently, the fall 2006 was dry (experiments with beetles were not conducted that time).

Generally, it is possible to say that average depth of entry holes was bigger on trees which were less stressed by a lack of water. The exact mechanism of these relationships between the water stress and the increase of tree resistance up to some level is not known yet (Reeve et al. 1995). We can only speculate that water stress is predisposing factor only after excess of some level, when defensive mechanism of tree is not able to produce the necessary amount of resin. Our results suggested that when trees suffer a low level of stress, they are able to increase their resistance and to be resistant longer time. In opposite, trees which were stressed and stress diminished due to late precipitation, became more attractive and less resistant. However, these speculations need to be confirmed by more extensive experiments.

CONCLUSIONS

The differences in attack rates and speed of entry holes preparation between differently positioned trees on slope were not statistically significant according to results.

The results of manipulated experiments indicated, that frequency and speed of boring was similar on more stressed versus less stressed trees during hot and dry weather, but frequency and speed became higher on less stressed trees in wet and colder period later.

These preliminary results suggested that the role of water stress is complicated and it is necessary to repeat manipulated experiments with higher number of inoculated beetles and on different position on the trees (on stem under canopy).

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Výsledky manipulovaných experimentů s inokulací *Ips typographus* (L., 1758) na smrcích s rozdílnou úrovní vodního stresu

ABSTRAKT: Ve smrkových porostech na severozápadě Slovenska byly realizovány manipulované inokulační experimenty se samci *Ips typographus* (L., 1758). Modelové stromy byly stresovány nedostatkem vody připravenými stříškami a na nich byly vedeny inokulační experimenty s lýkožroutem smrkovým. Na základě výsledků nebyly rozdíly mezi napadením stromů s různou pozicí na svahu statisticky významné (P = 0,389 pro stromy na bázi a ve středu svahu, P = 0,924 pro bázi a vrchol svahu a P = 0,530 pro střed a vrchol svahu, t-test). Rovněž rozdíly v napadení a rychlosti vytváření závrtů mezi více a méně stresovanými stromy nebyly statisticky významné (P = 0,321, t-test). Výsledky inokulačních pokusů indikují, že mírná hodnota vodního stresu nevede zákonitě ani ke zvýšení napadení, ani k rychlejšímu zavrtávaní samců do stromu. O získaných výsledcích se diskutuje.

Klíčová slova: *Ips typographus*; smrk; vodní stres; manipulované inokulační pokusy

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