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Physiological Method for Evaluation of Vigor State of Pine Stands

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Abstract

Vigor state of middle-aged pine stands was estimated during 2002-2008 at permanent sample plots by two methods: 1) visual estimation of stand vigor rank according to the 6-point scale of Forest Regulations of RF and 2) method of induction of tree defense response (injection of stem with high molecular extractives from *Ceratocystis laricicola* mycelium and registration of parameters of stem phloem necrosis). The stands of similar origin and structure were preliminarily divided into background and polluted ones on the basis of different amount of polluting substances in snow cover. Estimation of external tree signs according to 6-point scale didn't reveal significant differences between stands in monitored period. In contrast to this morphological method parameters of specific necrotic response of stem inner bark to fungal extract differed significantly in background and polluted stands. Parameters of the necrotic spots on the phloem were shown to depend on effect of creeping fire. The necrosis size increased and the necrosis "moved" toward crown during two years after the fire. The results are in accordance with the hypothesis that the size of phloem necrosis induced by the effect of extractives from phytopathogenic fungus mycelium on stem vascular tissues was determined by the tree state – it increased in damaged tree and decreased with tree state improvement. The injection method can inform about changes of physiological condition of tree and stand at the latent stage when exterior characteristics of a tree haven't changed yet.

Key words: Ecological factors, Extractives from *Ceratocystis laricicola* mycelium, Defense response, Size of necrosis of stem phloem (inner bark), Vigor state of pine stand

Introduction

The methods of trees and stands state estimation are mainly based on the visual control of the external signs or the measurements of the "internal" parameters (Alekseev, 1989; Sudachkova *et al*., 1997; Mikhailova and Berezhnaya 2002; Pavlov, 2006). Widely used visual estimation of vigor rank according to the 6-point scale of Forest Regulations of the Russian Federation (1998) doesn't allow us to avoid subjectivity (Abaturov, 2002) as well as has insufficient sensitivity for registration of early stages of change of plant vigor state. The analytically measured parameters (biochemical, physiological, histological, etc.) were sometimes difficult to be connected with actual tree and stand vitality. Development of effective methods for early detection of the stand state changes under the influence of the environmental factors is an important problem.

For evaluation of pine tree and stand state a method with application of fungal extractives was proposed, which turned out to be effective to recognize differences between background pine stands and polluted ones of similar ages and structure (Polyakov *et al.*, 2005). This method is based on the technique suggested in the middle of the last century that involves the stem inoculation with phytopathogenic fungi vectored by the xylophagous insects and is commonly used for investigations of host plant and pathogen relationships (Reid *et al*., 1967; Paine *et al*., 1997). The plant tissue responds to pathogen inoculation by quick necrotization – hypersensitive reaction. In case of successful defense response the pathogen was isolated in the boundaries of necrosis.

In our experiments on four conifer species the injection of stem by microdose of high molecular extractives from mycelia of different phytopathogenic fungi as well as inoculation with mycelia themselves showed to induce quick necrotization of the stem phloem (Polyakova *et al*., 2008). As a result of this defense response the necroses were formed in phloem, the size of the necrotic spot being more than that in the case of the control wounding. It gave evidence of participation of fungal metabolites in the induction of necrotic response. We suggested the size of this necrosis to be dependent on the tree state – increase in damaged trees and decrease when tree state improved and therefore it may be an indicator of tree vitality.

The purpose of this study was to verify the possibility to use the parameters of the necrotic response of the inner bark induced by stem injection with the fungus extractives for early diagnostics of change of stand state under the influence of different environmental factors. For that at the permanent sample plots in the suburban pine forests a comparison of two methods was carried out: 1) estimation of the stand vigor rank according to the 6-point scale of Forest

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Regulations of RF and 2) a method of induction of tree defense response by the fungal extractives.

Materials and Methods

The estimation of stand state by different methods was carried out at 5 permanent sample plots (SPs) in Krasnoyarsk suburban pine stands of similar age and structure but of different pollution degree (Fig. 1, Table). Plots 1-4 were established in 2002, SP 7 – in 2005. Each tree on the plots was numbered (Fig. 2).

Establishing of SPs (200-300 trees on the plot) was carried out according to operating Direction for forest management (1995). For each tree the stem diameter at 1.3 m was measured, for selected ones – the height (25-30 trees on each SP). Average stand diameter was calculated as root-mean-square-value, average height – by means of average diameter using logarithmic function.

Berezovsky pine forest is located 7 km windward off the town boundaries and strongly affected by industrial pollution (Tatarintzev, Skripal'shikova, 2003; Polyakov *et al*., 2005). In contrast to this, pine stands near Ovinnyi village being at the same distance but at the opposite side from the town do not experience such anthropogenic impact and so may be considered as background ones.

SPs 1, 2 and 7 are situated in Berezovsky pine forest, SPs 3 and 4 – in pine stands near Ovinnyi village. SPs 1

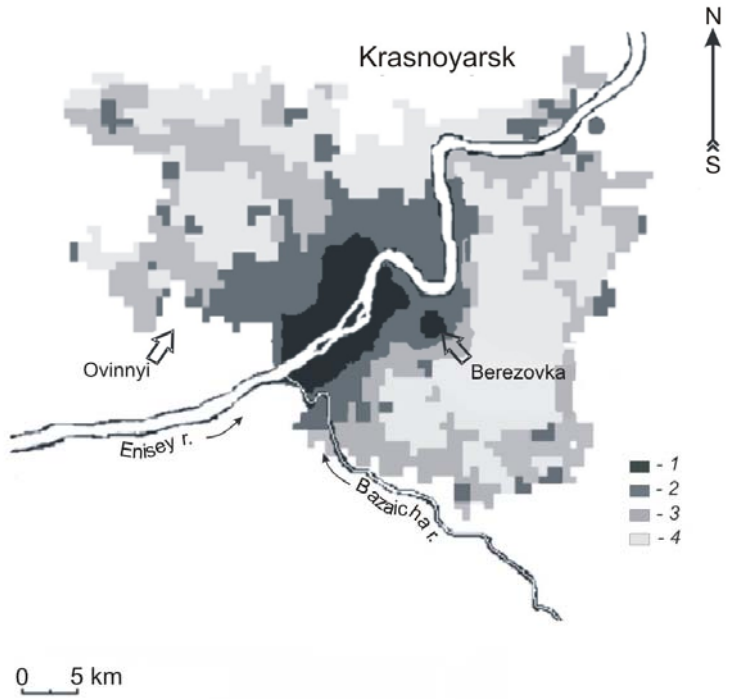

Fig. 1. Location of permanent sample plots: Berezovka Settlement (polluted pine forest), Ovinnyi village (background pine forest); contamination levels of suburban areas of Krasnoyarsk (Skripal'shicova *et al*., 2002): *1*, heavy; *2*, middle; *3*, moderate; *4*, weak.

Table 1. Inventory parameters of forest stands on SPs in 1 ha.

SP no.	Inventory years	Stand composition	Degree of density	Principal forest element	Basal area at 1.3m, m^2	Mean				Stock, m^3		Depth, ind.	
						age, years	vigour rank	height, m	diameter, cm	green timber	deadstanding trees	green timber	deadstanding trees
1	2002	10PII+PI	1.4	PII	45.0	57	I,6	18.1	18.5	396	6	1671	56
»	2008	10PII+PI	1.5	»	48.8	63	I,4	19.4	19.9	456	6	1566	63
2	2002	9PII+PI rB	1.5	PII	48.7	58	I,4	21.3	21.9	489	10	1286	36
»	2008	10PII+PI rB	1.6	»	52.3	64	I,4	22.8	23.3	557	6	1221	57
3	2002	9P1B rL	1.5	P	46.7	62	I,3	21.1	20.4	472	8	1430	81
»	2008	9P1B rL	1.6	»	50.2	68	I,4	22.6	21.9	535	5	1333	74
4	2002	9P1L+B	1.5	P	50.0	62	I,4	20.9	19.6	503	9	1664	172
»	2008	9P1L+B	1.6	»	52.6	70	I,5	22.5	21.2	561	11	1492	133
7	2005	9PII1PI	1.5	PII	41.3	59	I,5	16.3	16.3	333	3	1980	41
»	2008	9PII1PI	1.5	»	43.0	62	I,5	16.8	16.8	357	3	1932	47

Notes: PI and PII, pines of the first (parent) and second (daughter) generations, respectively; B, birch; L, larch; 10, 9, 1, +, r, proportion of the species (forest element) in the stand determined from the green timber stock: 100, 90, 10, 2-5, and below 2%, respectively; diameter, trunk inventory diameter at a height of 1.3m.

and 7 represented the most polluted sites of windward forest edge. SP 2 – less polluted area in the middle of the forest. Anthropogenic impact at SP 7 is aggravated by closeness of trunk-road with heavy traffic. Pine stand at SP 2 has forest inventory parameters close to ones of background stands at SPs 3 and 4 (Table). Decreasing of tree sizes and forest timber stock were registered at SP 1 and especially SP 7.

In spring 2004 an intensive creeping fire passed through the background stands at SPs 3 and 4. The height of carbon deposition on tree stems runs up to 2 m. There were no fires of similar intensity at other SPs during 2002-2008.

In 2006-2007 in monitoring area at SPs the essential increase of rainfalls was noted in comparison with annual average rate of several previous decades (State Report…, 2008).

Fig. 2. Numbered trees at permanent sample plot in the background pine stand.

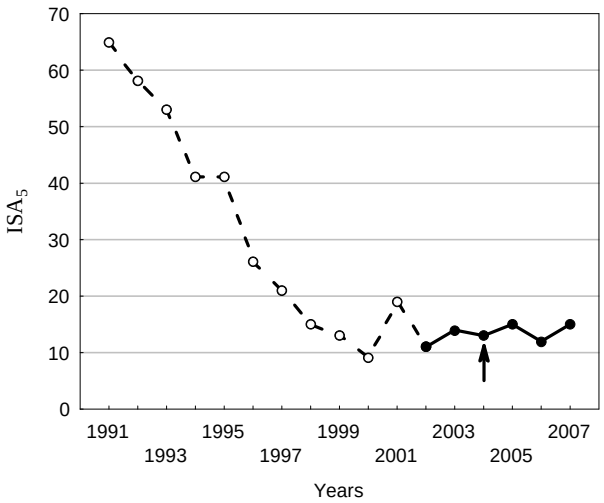

Fig. 3. Index ISA_5 in Krasnoyarsk before (dotted line) and in the period (firm line) of monitoring 2002-2008. Arrow marks creeping fire in the background stands in 2004.

For estimation of pollution level we used index ISA_5, calculated in relative numbers for five priority types of pollutants (Fig. 3) (State Report…, 2008). If this index is less than 5 the pollution is considered to be low, if it is over 14 – high. For atmosphere of Krasnoyarsk city ISA_5 decreased many times in the 90s and during the monitoring period – from 2002 up to now – ranged 11-15 without explicit tendency to change.

For quantification of pollutant content in snow cover of background and polluted stands in late March 2002 the snow cores were cut by cylindrical snow sampler from the snow surface to the ground. Precipitation after melting was dried and weighed.

Every year the vigor rank for every tree on SPs was determined according to the Forest regulations of RF(1998). The average vigor rank for stand was calculated as a weighted average for trunk volumes. Rank 1 includes trees without weakening signs, 2 – weakened trees, 3 – considerably weakened trees, 4 – suppressed trees, 5 – dead trees of current year, 6 – dead trees of previous years.

Every year at the stems of the sampled trees (25-30 trees per SP) the round holes of 7 mm diameter were made in inner bark to sapwood. These holes were injected with 0.5 mg of high molecular weight fraction of extractives from mycelium of phytopathogenic fungus *Ceratocystis laricicola* Redfern&Minter (Fig. 4). This fungus is able to develop in vascular tissues of conifer stems after artificial inoculation (Polyakova *et al.*, 2008). Injection of mentioned dose of fungus extractives is sufficient for formation of necroses, which exceeded the sizes of control ones produced after punching 7 mm hole in inner bark.

The culture of *C. laricicola* was isolated from larch stem bark, damaged by *Ips cembrae* L. in 1998. The culture was maintained under 4°C on 2% malt extract agar. To obtain fungal biomass the method of batch culture without shaking was used. The fungus mycelium was cultivated in liquid 2% malt extract (total vessel volume – 1000 ml, volume of medium – 150 ml). The fungus 7-day-old culture on 2% malt extract agar was used as inoculum.

Fig. 4. Injection of stem with fungal extractives using a micropipette.

Mycelium was crushed in liquid nitrogen, homogenized by ultrasound, extracted with ethanol; high molecular fraction was isolated by dialysis. Four weeks after injection the vertical length of necrosis looking like oblong ellipsoid along the stem was measured (Fig. 5). This period was sufficient for formation of dimensionally stable necrosis. Average necrosis size for the stand was calculated as average weighted to stem volume.

To estimate vertical asymmetry of necrotic spots the length of upper part of necrosis was measured - i.e. distance from the centre of inoculation hole to the upper boundary of the spot.

Collection of long-term monitoring data at SPs and parameter analysis were carried out in database in MS Access.

Fig. 5. Necrotic spot on inner bark surface 4 weeks after injection of 0.5 mg extractives from *C. laricicola* mycelium.

Results

Stressed ecological situation in Berezovsky forest in comparison with background stands testified 7-hold increasing of contaminant contents in snow cover – 37.5±3.7 and 5.4±0.7 g/m^2 correspondingly.

During monitoring period at SPs the average vigor rank in the background and the polluted pine stands didn't differ significantly (at 5% level), but at the same time the improvement of vigor rank of polluted stands and worsening in background ones were noted as tendencies (Fig. 6, left).

Average necrosis size in polluted stands at the monitoring start was significantly more than in background ones (Fig. 6, right). After fire in 2004 the average length of the necrosis in the background stands increased significantly during two years and from 2006 sizes of necroses were larger in comparison with the necrotic spots in the polluted stands.

After the fire of 2004 in background stands the two-year period of reliable "asymmetry" of necrotic spot upward the stem was registered: the length of the upper part of necrosis exceeded essentially the size of its lower part (Fig. 7).

Discussion

The observed tendency of improvement of Berersovsky forest vigor may be caused by effect of two factors: 1. Gradual consequences of multiple decreasing of the pollution index ISA_5 during the 90s (Fig. 3). 2. Registered by meteorological service significant increasing of rainfall in the vegetation seasons 2006-2007 that favored to needles surface ablution, gas exchange intensification and functioning of photosynthetic apparatus. Our observations are in good agreement with the published data. Reduction of industrial emissions caused by setback of production in Shelekhovsky industrial centre corresponded to pine

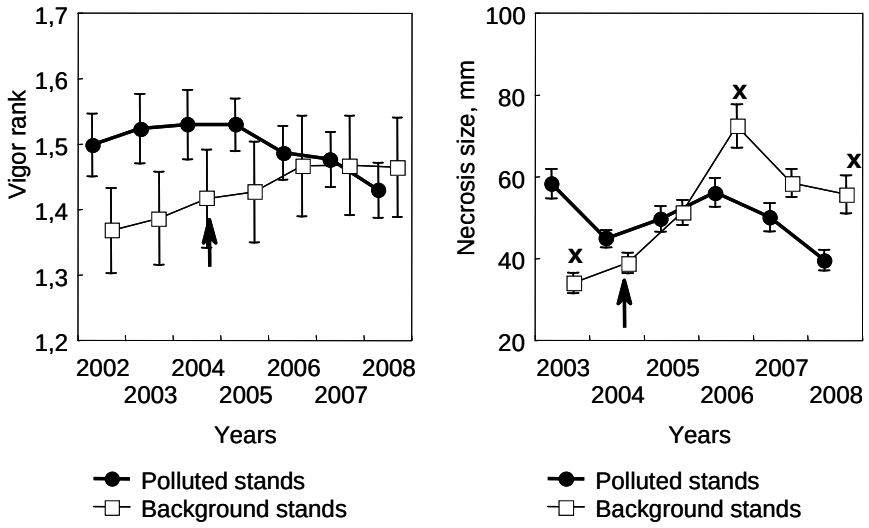

Fig. 6. Average vigor ranks and necrosis sizes on inner bark 4 weeks after injection of stem with fungus extractives. Symbol "x" marks values differed in background (light markers) and polluted (black markers) stands (P>0.95), arrow – fire effect.

stands state improvement 5-7 years after pollution decrease (Mikhailova and Berezhnaya, 2002).

Stimulating effect of low "before-threshold" concentrations of contaminants resulting in increase of radial increments of conifer species, photosynthetic rate and chlorophyll content (Pavlov, 2006, p.77) corresponded to nonspecific adaptation syndrome of plants (Pakhomova, 1995). According to this conception an adaptation process is characterized by active counteraction to aggressive environment, maximization of defense functions of plant physiological systems directed to overcoming of stress factor consequences. If stressor's effect was too long and intensive, phase of resistance changes into phase of depletion and consequent plant death that are observed in zones of stand decline near large industrial centers (Kharuk *et al.*, 1996; Mikhailova and Berezhnaya, 2002).

In Berezovsky pine forest the depression of the phloem annual increment was found, which was 1.7 times less in comparison with the background parameter (P>0.95) (Immune response…, 2008, p.80). It was an evidence of a stressed state of the polluted stand.

The absence of significant, at 5% level, differences of the vigor ranks of the background and the polluted pine stands under the influence of various environmental factors (pollution, rainfall increase, fires) is evidence of insufficient sensitivity of this characteristics for registration of effect of the environmental factors on the stands state.

"Necrotic" response of tree to fungal extract appeared to change in period of monitoring significantly. After creeping fire in 2004 in the background pine stands the two-year period of significant necroses increasing was registered, whereas increase of vigor rank was revealed as a tendency only (Fig. 6).

Increase of necrosis sizes indicates the intensification of diffusion of fungal metabolite or another response triggering signal into vascular tissues of tree stem. Possible cause of the necrotic zone expansion appears to be disturbances of defense transformation of phloem cell walls induced by tree weakening by high temperatures. Previously it was shown that in pine the defense response to stem inoculation with extractives from fungus *C. laricicola* was accompanied by lignin accumulation in sieve cell walls (Immune response…, 2008, p. 62). Our study showed that induction of sieve cell lignification after our treatment was registered up to the distance of 30-40 mm from the necrosis spot boundary (unpublished data).

The method seems somewhat to be destructive for inner bark. But in our long-term study (during 20 years) we never observed visually any sign of other fungi infection. Perhaps this is due to the fact that stem area for measurements of necrotic spot dimensions where outer bark was removed and surface of inner bark might be damaged was considerably less than area where content of lignin is found to be increased as a result of induced response. The lignification is known to be an important factor of anti-infectious defense (Vance *et al.*, 1980).

The registered two-year post-fire period of significant "shift" of necrotic spot upward stem (Fig. 7) appeared to be caused by disturbance of normal basipetal transport of photoassimilates as a result of needle photosynthetic apparatus damage by high temperatures at the height of tree crown and necessity of needles regeneration. These data appeared to show the influence of assimilate transport upon intra-stem diffusion of fungal metabolites or another signal which induce necrotic process in stem inner bark.

Long-term observations in pine stands showed the

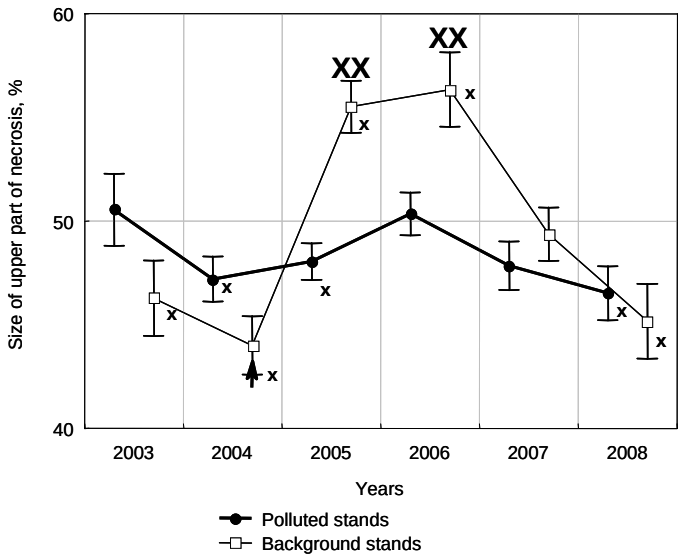

Fig. 7. Average size of the upper part of necrosis of inner bark 4 weeks after injection of stem with fungal extractives. Symbol "XX" marks values differed in background (light markers) and polluted (black markers) stands (P>0.95), symbol "x" indicates significant differences between the upper and the lower parts of the necrosis, arrow – fire effect.

tendencies of average vigor rank changes: improvement of state in the polluted stands and worsening – in the background ones (Fig. 6, left). The dimension of the necrosis at the beginning of the monitoring period was essentially more in the polluted stands than in the background ones, but from 2006 the mean necrosis length in Berezovsky pine forest became less (Fig. 6, right). These results are in accordance with our hypothesis that the size of the phloem necrosis induced by the effect of high molecular extractives from phytopathogenic fungus mycelium on stem vascular tissues depended on the tree state – it increased in damaged tree and decreased with tree state improvement.

Thus, the study showed that the size of the inner bark necrosis induced by injection of fungal extractives into stem vascular tissues responded to changes of tree vigor in connection with environmental situation. The parameters of necrotic response of stem inner bark to fungal extract differed significantly in the background and the polluted stands. Parameters of necrotic spots were shown to depend on effect of creeping fire. The results obtained showed a possibility to use the method of induction of defense response for early diagnostics of changes of state in pine stands. The injection method may give information about changes of physiological condition of tree and stands at the latent stage when exterior characteristics of tree (vigor rank by 6-point scale) haven't changed significantly yet.

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