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ORIGINAL ARTICLE

Dynamics of *Pinus sylvestris* L. needles activity in Predbaikal'ye forests

I.M. Romanova, M.A. Zhivet'yev, T.A. Penzina, I.A. Graskova

Siberian Institute of Plant Physiology and Biochemistry SB RAS, Irkutsk, Russia

*E-Mail: ***graskova@sifibr.irk.ru***

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The study was aimed to analyse changes in peroxidase activity of common pine needles throughout the year. The needles of the 1-st, 2-nd and 3-d years were collected. Total activity of guaiacol-dependent peroxidase was determined. Minimum peroxidase activity is observed in January and February. In March intense overall activation of peroxidase is observed in the needles of all ages, which might be related to spring enhancement of physiological processes and activation of metabolism. Autumn period is characterized by more or less high activities of guaiacol peroxidase from September till November, which is related to its participation in stress-adaptation to low temperatures in winter. Peroxidase activity changes depending on the season of the year. Needles of different age are characterized by their own peroxidase activity level in various time periods.

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Pinus sylvestris is one of the major forest-forming species in the Prebaikal'ye territory. In Siberia throughout the year it is exposed to intense change of temperature, which should affect the functioning of its enzyme systems in different years and phases of development, as the environmental temperature is the major limiting factor of plant growth and development, particularly in winter. One of the enzymes, which respond most to environmental conditions change, is peroxidase. Availability of multiple forms of the enzyme allows it to operate in diverse conditions and carry perform various functions. Thus, changes of activity and iso-enzyme spectrum of peroxidase are observed under various biological, physical and chemical impacts on plants, including temperature stress, chemical pollution of environment and plant introduction into new ecological conditions (Müftügil, 1985; Siesko *et al*., 1997; Gülen, Eris, 2004; Thongsook, Barrett, 2005; Gülen *et al*., 2008; Zolfaghari *et al*., 2010). Therefore, the present study was aimed at the analysis of changes in peroxidase activity in common pine *Pinus sylvestris* L. needles during the year.

MATERIALS AND METHODS

The research was conducted in the areas of the Bol'shoi Lug settlement, IrkAZ, Olkha settlement and Melnichnaya Pad' settlement. Samples were collected on a monthly basis from November 2011 till September 2012 inclusively. Needles of the 1-st,

2-nd and 3-d years were collected, and starting from July 2012 young (new) needles were collected. In the samples total activity of guaiacol-dependent peroxidase was determined. A sample (1 g) of the tissue was placed in 10 ml of cold citrate-phosphate buffer and pulverized in porcelain dish at 4 °C. The acquired homogenate was centrifuged at 3 th. rotations per minute during 15 minutes. The supernatant was used for determination of the enzyme activity (Граскова, 2004).

The activity of soluble peroxidases in plant leaves was determined by the change of optical density (wave length - 580 nm) in the reaction mixture of the following compositions: 0,5 ml 0,1 M of cold citrate-phosphate buffer (pH from 4,0 to 7,0 with the interval of 0,2), 0,5 ml 0,3 % of hydrogen peroxide («Reachim», Russia), 0,5 ml 0,05 % guaiacol (Sigma, the USA) and 0,5 ml of the sample (1g of the sample was pulverized in 10 ml of citrate-phosphate buffer pH 5,5).

Peroxidase activity was determined at 25° C immediately after the extraction of the enzymes from the samples. The enzyme activity was expressed in conventional units per 1 mg of raw weight of the tissues as per the formula:

$A = \sum (\alpha\ \beta\ \gamma)/d\ t$, where - extinction (0,125), α – proportion of the amount of buffer taken for preparation of the extract, in ml to the raw tissue weight, β – degree of additional dilution of the extract in the reaction mixture, γ – degree of constant dilution of the extract in the reaction mixture, d – thickness of the absorbing layer of the cuvette, t – reaction time (Бояркин, 1951).

RESULTS AND DISCUSSION

Minimum of peroxidase activity is observed in January-February, which is characteristic of the needles of all ages and all locations (Fig. 1-4).

This may be related to the plants being in forced peace state. In March intense omnipresent activation of peroxidase in the needles of all the three years is observed, which might be accounted for by spring intensification of physiological processes in the needles and activation of metabolism due to the switch from winter slow metabolism to active spring-summer metabolism. In April the activity of the enzyme decreases, but in May, before the emergence of young needles, a new peak of peroxidase activity is observed, with new sprouts appearing and intensely growing, except for the needles of 3-d year near IrkAZ (Fig. 1), when peroxidase activity started to grow not in May, but in July.

The data acquired presume that from November till July needles of the 1-st year near IrkAZ was characterized by higher peroxidase activity as compared to the needles of the 2-nd year, the activity values were even lower for the needles of the 3-d year. The above allows us to suppose the oppression of enzyme in pine needles in this sector. At the same time, with new needles appearing in June-July, intense growth of peroxidase activity is observed in the needles of the 3-d year, and this growth was twice as high as the increase of the activity of the 1-st year needles, whereas the second year needles increased its activity only by August (IrkAZ). For Melnichnaya Pad' (Fig. 2) the second year needles rank top by the peroxidase activity from November till May as compared to the needles of other generations. In Olkha (Fig. 3) this enzyme activity prevails in the third year needles throughout the whole year, except May, when the first year needles peak.

The highest enzyme activity values over the whole monitoring period are found for Bol'shoi Lug (Fig. 4) (the first year needles – in June, the second

year needles – in May, the third year needles in July), slightly lower values characterize Olkha (the first year needles demonstrate maximum activity peak in May, the second and third year needles – in March), even lower values are observed at IrkAZ (the first year needles demonstrated the highest activity in May, the second year needles – in March, the third year needles – in July). The lowest maximum peroxidase activity was found in Melnichnaya Pad' (the second an third year needles peaks are in May, the new needles peak is in July, and in the first year needles the activity fluctuates at a relatively low level with summer activity peaks not exceeding the winter peaks). This presupposes that in Bol'shoi Lug, Olkha and IrkAZ plants may be subjected to some stressing factors, unlike Melnichnaya Pad' area, where enzyme activity is kept at a relatively low level.

The autumn period is characterized by more or less high activities of guaiacol-dependent peroxidase from September till November-December, which is related to its participation in stress-adaptation to low temperatures in winter.

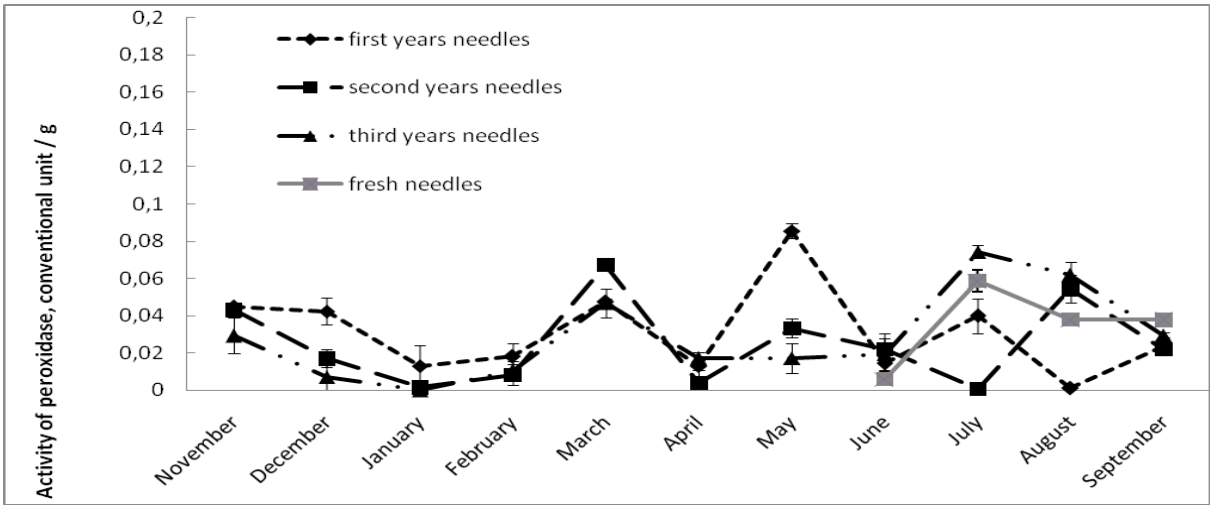

Figure 1. Annual dynamics of the activity of quaicol peroxidase in common pine needles, 2011-2012, IrkAZ: 1-November, 2-December (2011), 3-January, 4-February, 5-March, 6-April, 7-May, 8-June, 9-July, 10-August, 11-September (2012)

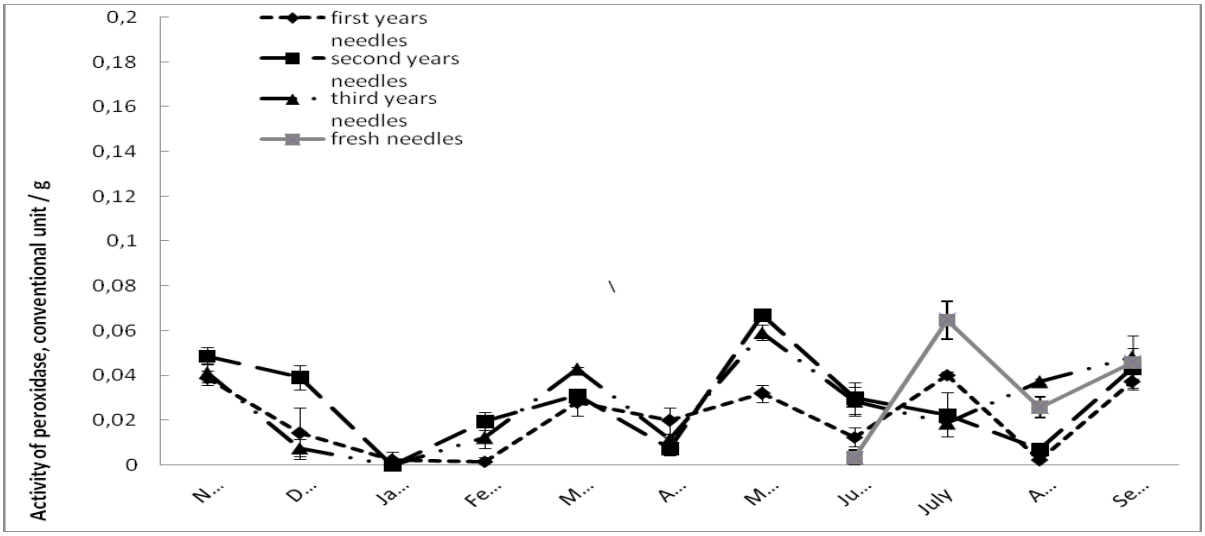

Figure 2. Annual dynamics of the activity of quaicol peroxidase in common pine needles, 2011-2012, Melnichnaya Pad': 1-November, 2-December (2011), 3-January, 4-February, 5-March, 6-April, 7-May, 8-June, 9-July, 10-August, 11-September (2012)

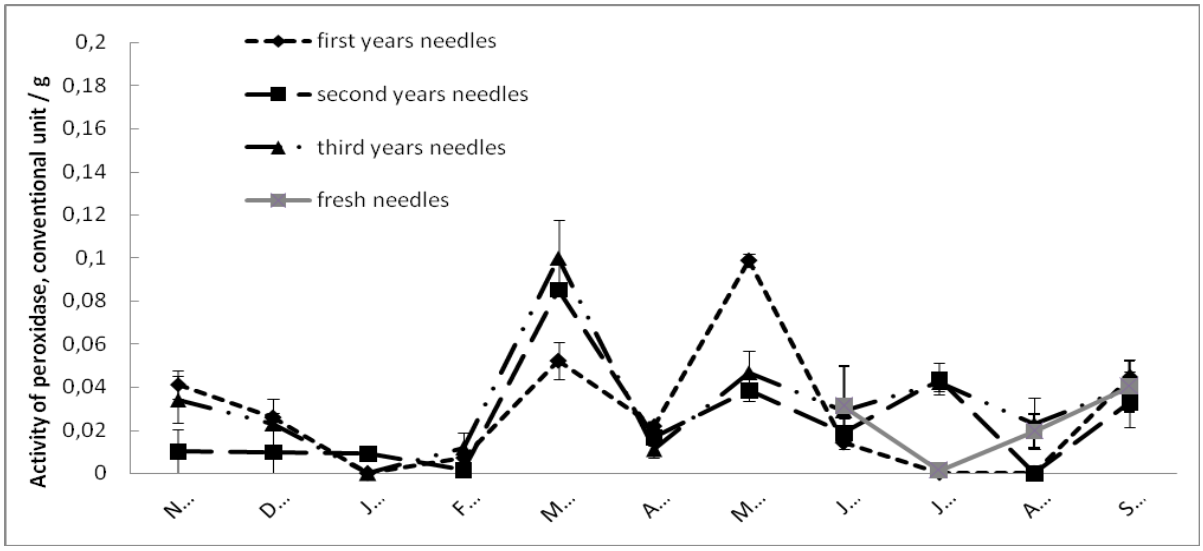

Figure 3. Annual dynamics of the activity of quaicol peroxidase in common pine needles, 2011-2012, Olkha: 1-November, 2-December (2011), 3-January, 4-February, 5-March, 6-April, 7-May, 8-June, 9-July, 10-August, 11-September (2012)

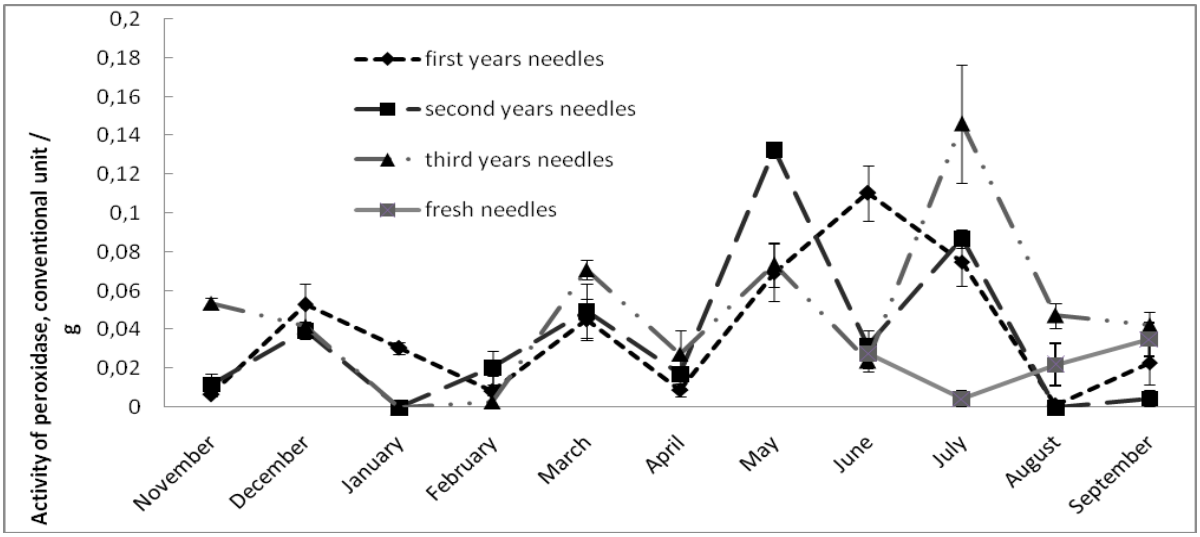

Figure 4. Annual dynamics of the activity of quaicol peroxidase in common pine needles, 2011-2012, Bolshoi Lug: 1-November, 2-December (2011), 3-January, 4-February, 5-March, 6-April, 7-May, 8-June, 9-July, 10-August, 11-September (2012)

CONCLUSIONS

Thus, the peroxidase activity in common pine was shown to change depending on the season of the year, which, most probably, is due to the temperature mode and the activity of plant organism vital functions. Needles of different ages are characterized by their own level of peroxidase activity in different time periods. There are observed significant differences in peroxidase activity dynamics in various selection points with different temperature mode, precipitation amount and technogenic load.

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