FORMATION OF NONSPECIFIC RESISTANCE AND INCREASE OF BIOPRODUCTIVITY OF SCOTS PINE SEEDLINGS UNDER TREATMENT WITH SEDAXANE

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Introduction. The full-scale military invasion of Russia on the territory of Ukraine brought great environmental and economic losses to forestry. Significant areas of forest plantations were affected by forest fires, damage of trees by bullets and shrapnel as a result of military operations in a large area along the entire line of combat clashes. In addition, a large number of forests were uncontrollably cut down for the needs of warfare - the construction of military defense constructions, bridges and water crossings, heating of military premises and shelters. Also, a large area of forests was destroyed in the Kherson and Mykolaiv regions as a result of Russian terrorist actions from blowing up the dam of Kakhovka Reservoir. Currently, it is impossible to calculate the damage in the forests of Ukraine until the end of the war. But in the first few years in the post-war period, the need for high-quality planting material to restore destroyed forest plantations will increase many times over in short period of time. Also, partially damaged plantations will require reforestation in the coming decade. Therefore, the question of effective cultivation of planting material with the use of the modern drugs to protect young seedlings of woody plants from various negative factors for further successful forest restoration in the post-war period is acutely facing the forest science and forestry production of Ukraine.

It is well known that millions of young plants die every year on forestry sites due to infectious diseases, which leads to significant losses. The most common and dangerous diseases of conifers in the nurseries of Ukraine is infectious root rot disease caused by fungi of the genera *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Verticillium* spp., *Botrytis* spp. etc (Karpets et al., 2014; Damszel et al., 2021; Gomdola et al., 2022). 30-45% of plants can die under the significant infectious damage, and in some cases – 85-100% (Elvira-Recuenco et al., 2019; Luo, Yu, 2020; Gomdola et al., 2022; Li et al., 2023). Also, the impact of adverse climatic factors, in particular drought, causes significant losses when growing coniferous seedlings, including pine (Steckel et al., 2020; Brown et al., 2020). Despite the relative tolerance of pine to drought, its sensitivity to this factor in the early stages of development is quite high (Steckel et al., 2020).

One of the effective techniques for protecting young seedlings of woody plants from biotic and abiotic factors can be the use of drugs based on Sedaxane as an active substance (Brown et al., 2020; Tong et al., 2022). Sedaxane (a mixture of *trans*- and *cis*-isomers of N-[2-(1,1'-bicyclopropyl)-2-ylphenyl]-3-(difluoromethyl)-1-methyl-1-H-pyrazole-4-carboxamide) – artificial a synthesized substance with pronounced fungicidal properties of contact action (Ebbinghaus et al., 2010; Jeschke, 2016; Dal Cortivo et al., 2017). The first information about the possibility of its use as a fungicide appeared about 10 years ago (Walter et al., 2015; Sharma et al., 2021). Currently, Sedaxane is used as one of the active substances in the disinfectants of Vibrance group produced by Syngenta (Lamberth, Dinges, 2016; Polson et al., 2020). It is believed that the main mechanism of action of Sedaxane is related to highly specific inhibition of succinate dehydrogenase of fungi and blocking of energy metabolism (Jeschke, 2016; Tong et al., 2022). Although this substance is positioned as a fungicide of contact action, there is information about its systemic effect due to the possibility of absorption by some plant organs and transport through conductive tissues to other organs (Lamberth, Dinges, 2016; Kolupaev et al., 2017; Steckel et al., 2020). It was established that Sedaxane is capable to inhibit plant succinate dehydrogenase, which has a certain structural homology to the identical fungal enzyme (Kolupaev et al., 2017; Shkliarevskyi et al., 2019; Sharma et al., 2021).

Currently, Sedaxane is considered as a physiologically active substance that can positively affect the resistance of agricultural plants to biotic and abiotic stressors (Dal Cortivo et al., 2017; Kolupaev et al., 2017; Shkliarevskyi et al., 2019). However, the physiological effects of Sedaxane on woody plants, including conifers, have not been investigated so far. Preparations based on it are recommended for use in Ukraine only for agricultural plants (State …, 2024).

In connection with the above, the aim of the work was to study the effect of pre-sowing treatment with Sedaxane on the resistance of Scots pine (*Pinus sylvestris* L.) seedlings to biotic (infectious root rot disease) and abiotic (artificial drought) stressors in laboratory soil culture.

Material and methods. Scots pine seeds were sown 300 seeds each in plastic cuvettes with sandy forest soil. Seedlings were grown at a temperature of 20±2°C, relative air humidity of 60±10%, illumination of 6 klk (photoperiod of 14 h) with moderate daily watering to maintain the relative humidity of the substrate at the level of 70-80% of the full moisture capacity (Karpets et al., 2014; Karpets et al., 2017).

Pre-sowing treatment with Sedaxane was carried out by immersing the seeds in appropriate solutions in the concentration range of 0.001-10 g/l for 1 h, followed by drying on filter paper before sowing (Shkliarevskyi et al., 2019).

The number of seedlings unaffected by the causative agent of the infectious root rot disease was found by continuous counting in each biological replication. The height was calculated as the arithmetic mean value of the measurements of 30 seedlings or, if the remainder is smaller, all the seedlings of biological replication. The number of unaffected seedlings and their height were determined starting from the 20th day after sowing the seeds in the soil with an interval of 10 days until the 60th day of observation, after which, as a rule, infectious root rot disease stops due to the beginning of intensive lignification of pine seedling tissues (Karpets et al., 2014).

An artificial drought was created for 10 days, starting from the 20th day after sowing the seeds, by reducing the intensity of watering with the gradual decrease in the relative humidity of soil to 25-30% of the full moisture capacity (Karpets et al., 2017).

When determining the wet and dry mass of the above-ground part of plants and water balance indicators, 50 seedlings were selected from each biological replication. To determine the water deficit, the plant material was placed in cuvettes with distilled water for 2 h without the access of light to completely saturate the tissues with water (Karpets et al., 2017).

Experiments were completed independently twice with four biological replicates each. Figures show mean values and their standard errors. The significance of the differences was assessed by Student's t-test. A difference at $P \leq 0.05$ was considered significant, a difference at the level of trends – at $P \leq 0.1$.

Results. *Growth effects of Sedaxan*. Seed treatment with Sedaxane in concentrations ranging from 0.001 to 1 g/l increased soil fertility, which was assessed on the 10th day after sowing (Figure 1). A concentration of 10 g/l significantly inhibited seed germination.

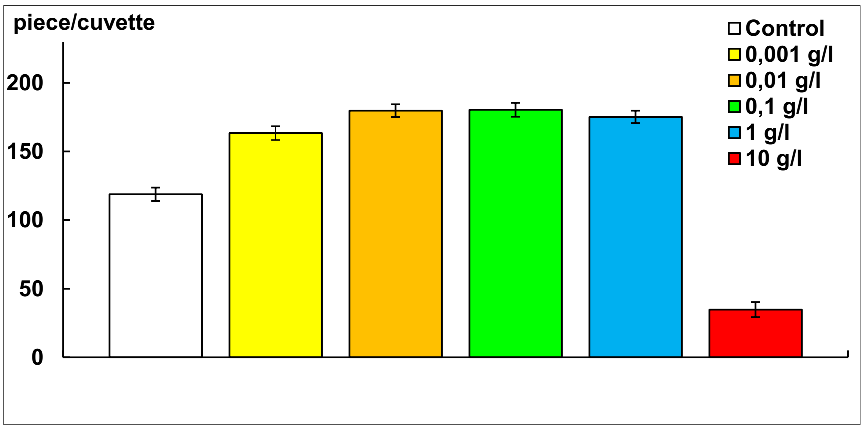


Figure 1. The effect of Sedaxane on the soil germination of Scots pine on the 10th day after sowing.

Under the influence of Sedaxane in concentrations from 0.001 to 1 g/l, the growth of pine seedlings increased in height (Figure 2). The difference between the experimental and control variants was especially noticeable at the beginning of the experiment, then this effect decreased somewhat, but remained significant. The concentration of 10 g/l of Sedaxane had a negative effect on seedling growth throughout the experiment (Figure 2).

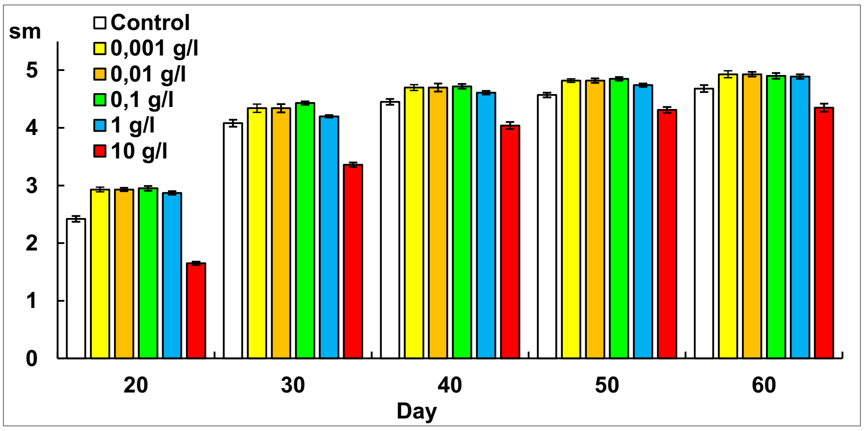


Figure 2. The effect of Sedaxane on the height growth of Scots pine seedlings.

In variants with seed treatment with Sedaxane in non-toxic concentrations, a tendency to increase the fresh weight of seedlings was noted, while a significant positive effect of Sedaxane in variants with concentrations of 0.01, 0.1 and 1 g/l on the accumulation of dry biomass was established (Figure 3).

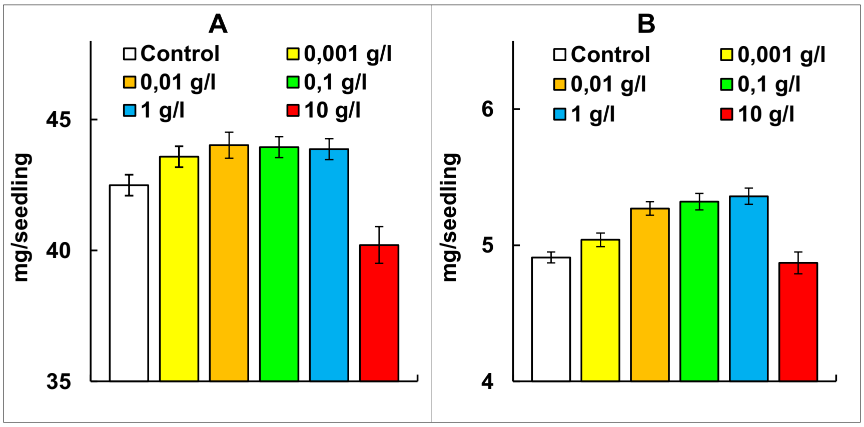


Figure 3. The effect of Sedaxane on the accumulation of fresh (A) and dry (B) mass of Scots pine seedlings.

Effect of Sedaxane on resistance of seedlings against infectious root rot disease. A significant increase in resistance against infectious root rot disease compared to the control was found in variants with Sedaxane in concentrations of 0.01, 0.1 and 1 g/l (Figure 4). The concentration of 0.001 g/l had a relatively small positive effect, and 10 g/l showed the greatest protective effect against fungal lesions, although against the background of suppression of seed germination and growth of seedlings in height (Figs. 1-3). At optimal concentrations of Sedaxane (0.01, 0.1, and 1 g/l), which did not suppress seed germination and seedling growth, their preservation in the end of experiment was 6-7 times higher compared to the control (Figs. 4, 5).

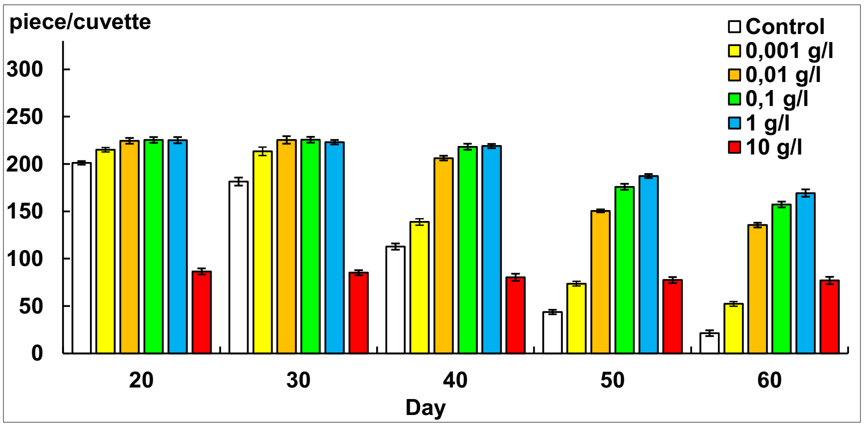


Figure 4. The influence of Sedaxane on the damage of Scots pine seedlings by root rot disease.

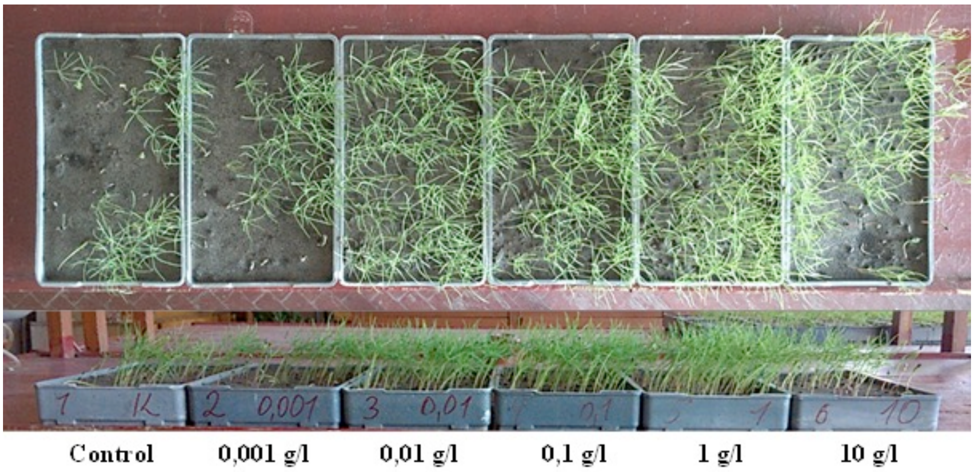


Figure 5. The state of Scots pine seedlings under the influence of Sedaxane on the 60^{th} day of the experiment on the study of inducing resistance against root rot disease.

The effect of Sedaxane on the resistance of seedlings to soil drought. Under conditions of artificial drought, the positive effect of Sedaxane on the linear growth of seedlings was manifested in concentrations from 0.001 to 1 g/l (Figure 6). The highest concentration of 10 g/l alone inhibited growth in height (Figure 1), but significantly contributed to its preservation under drought conditions (Figure 6). At the same time, Sedaxane concentrations from 0.01 to 10 g/l showed a visible positive effect on turgorescence of seedlings (Figure 7).

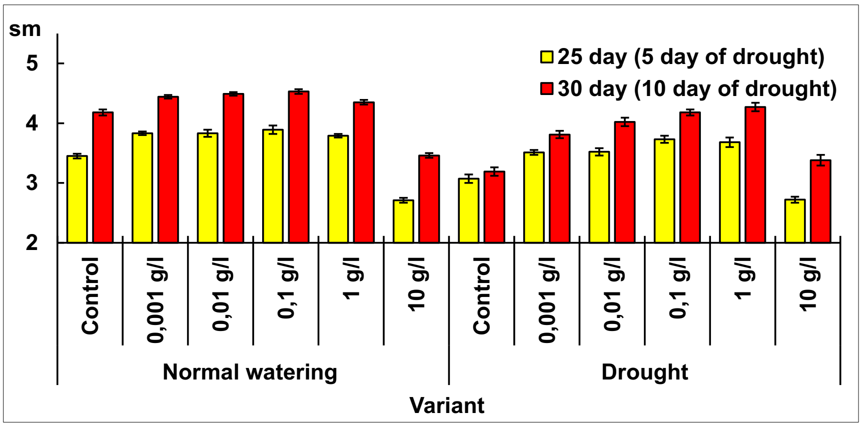
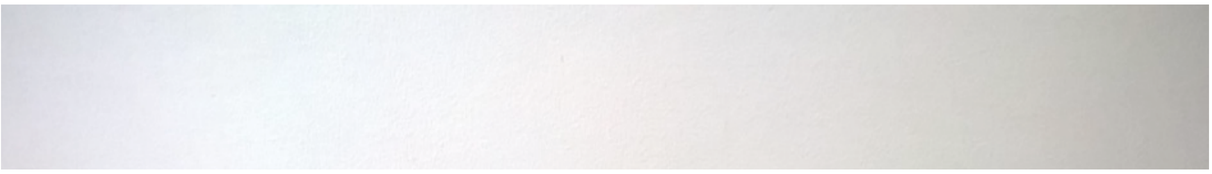


Figure 6. The effect of Sedaxane on the height growth of Scots pine seedlings under normal watering and under the influence of drought.



Control norm. water. **Control** **0,001 g/l** **0,01 g/l** **0,1 g/l** **1 g/l** **10 g/l**
Drought

Figure 7. The state of Scots pine seedlings at the end of a 10-day drought.

Sedaxane in all non-toxic concentrations had a positive effect on the accumulation of fresh and dry masses (Figure 8). Treatment of seeds with Sedaxane in concentrations of 0.01-1 g/l increased the hydration of seedlings (Figure 9, A). Under the influence of Sedaxane in the entire range of concentrations, a decrease in the water deficit index was noted under drought conditions, while such a decrease was proportional to the value of the used concentration (Figure 9, B).

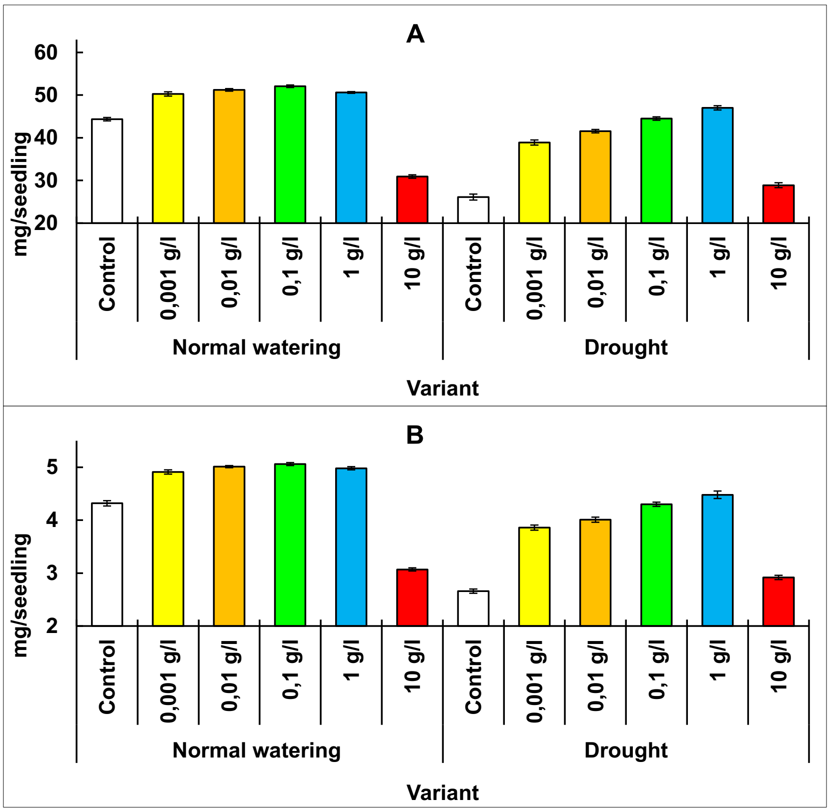


Figure 8. Effect of Sedaxane on the fresh (A) and dry (B) mass of the above ground part of Scots pine seedlings at the end of 10-day drought.

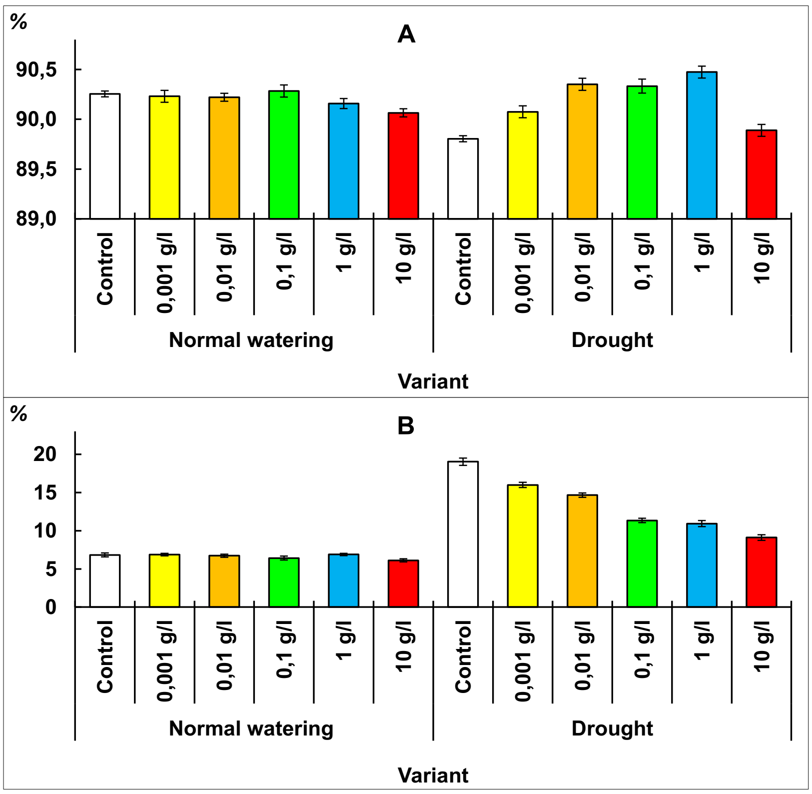


Figure 9. The effect of Sedaxane on the hydration (A) and water deficit (B) of Scots pine seedlings under the normal watering and under the influence of 10-day drought.

Discussion. The conducted studies showed a positive effect of Sedaxane both on the resistance to the causative agents of infection (actually, a fungicidal effect), and on the growth of uninfected plants and their resistance to soil drought. So, it can be stated that its effects are not limited to fungicidal action.

In separate studies conducted on herbaceous agricultural plants, data were obtained indicating the manifestation of physiological activity of Sedaxane in relation to plants (Tong et al., 2022; Dal Cortivo et al., 2017; Walter et al., 2015; Kolupaev et al., 2017; Shkliarevskyi et al., 2019). In particular, the growth effects obtained on corn plants allow us to ascertain the presence of Sedaxan, at least phenomenological manifestations of auxin and gibberellin activity (Dal Cortivo et al., 2017).

Another reason for the positive effect of Sedaxane on plants may be its modification of succinate dehydrogenase activity. It was established that Sedaxane can affect not only the succinate dehydrogenase of fungi, but also plants enzyme of different taxonomic affiliations (Kolupaev et al., 2017; Shkliarevskyi et al., 2019; Tong et al., 2022). Under the influence of Sedaxane on wheat and corn seedlings, a decrease in the activity of succinate dehydrogenase, a decrease in the formation of hydrogen peroxide, and a decrease in the accumulation of the product of lipid peroxidation under conditions of osmotic stress were recorded in them (Kolupaev et al., 2017; Shkliarevskyi et al., 2019). It is possible that the inhibition of succinate dehydrogenase under stress conditions can lead to the reduction in development of oxidative stress associated with the functioning of mitochondria.

Another component of the positive effect of Sedaxane on plants under stress conditions may be its ability to activate enzyme phenylalanine ammonium-lyase and, as a result, to increase the accumulation of phenolic compounds (Dal Cortivo et al., 2017). As it is known, these compounds have antioxidant properties (Kolupaev, Karpets, 2019). In addition, they can participate in strengthening cell walls, which is important for the resistance of plants to both abiotic and biotic stressors.

Conclusions. Thus, in this research, the growth-stimulating and stress-protective effects of Sedaxane on young Scots pine plants were revealed for the first time. An increase in the resistance of seedlings to infectious lodging has been established by treating seeds with Sedaxane in concentrations of 0,01-1 g/l. Under drought conditions, pre-sowing seed treatment with Sedaxane in the same concentration range had a positive effect on plant growth and water balance. The obtained phenomenological data are the basis for further studies of the influence of Sedaxane on redox processes, primary and secondary metabolism of compounds associated with the response of woody plants to the action of stress factors.

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