

Current possibilities and prospects of using fungicides in forestry

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Abstract. The possibility of using chemicals in European forestry is extremely limited due to the binding legal regulations and specific conditions concerning the market of plant protection products. This is reflected in the limited availability of active fungicides in forestry. Due to this limitation, practitioners using fungicides in forest nurseries and forest cultivation must have substantial knowledge of the biology of pathogens to ensure satisfactorily effective protection.

The work presented here provides an overview of the currently recommended fungicides in Polish forestry as well as the mechanisms of interaction between the active substances and the pathogen, the plant and mycorrhizal fungi. The risk of fungicide resistance, which has been insufficiently explored in the context of forest pathogens, is also discussed in this paper.

Keywords: forest protection, forest nurseries, fungicide resistance, fungicides mode of action

1. Introduction

Integrated protection of forest nurseries came into force by way of legal dispositions of the European Commission (Directive No 2009/128/WE dated on October 21, 2009 and the Regulation No 546/2011 dated on June 10, 2011 concerning the integrated plant protection against pests). The idea behind it consisted in the complementary usage of numerous (or all) possible plant protection methods. Therefore, the Ministry of Agriculture and Rural Development prepared a project of a national plan of action for the limitation of risk related to the use of plant protection chemicals for 2013–2017. Unfortunately, due to costly assessment processes concerning the influence of respective active substances on the environments (amounting to several millions of Euro) some of the producers resigned from taking the effort to register the plant protection chemicals. In result, the supply of the preparations available on the market decreased significantly. The forest practitioners have half the number of plant protection chemicals at their disposal. So what should be done in the event of limited choice of fungicides? This review lists preparations currently admitted to use in forestry and shows the mechanisms of their functioning and current

and potential effectiveness on different groups of pathogenic organisms, along with the simultaneous analysis of the possibility of fungicide resistance (organisms present in Poland and closely related to them).

2. Current state of chemical protection in forestry

The specificity of forest nursery cultivation causes that the risk of the existence of fungal pathogens menace for the saplings is considerably high, and at the same time, due to the minute range of cultivated plant species the accumulation of pathogenic factors may occur, while the limited stock of plant protection chemicals recommended for the use in forest nurseries brings about the situation where the fungicide treatment often proves ineffective. Currently the forest nursery uses a few fungicidally active compounds, (Fig. 1) which constitute an enormous challenge both for the practitioners and researchers whose task consists in the optimization of plant protection. This state of affairs is directly related to the policy of chemical companies, for whom high costs of the registration of fungicides in cultivations of small areas (such as nursery production) constitute a barrier blocking the introduction of innovative fungicides. Therefore nursery production most often

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takes advantage of the interventional fungicide treatment with the use of only a few active substances used for many years. Additionally, the necessity of repeating the treatment on the same plots of forest nurseries with active substances from the same group or of identical mechanism of interaction with the cells of fungi sanctions the pathogens resistant to the preparations. At the moment, the programme of the General Directorate of the State Forests of Poland is being implemented and concerns the intensification of the forest nursery production, where greater problems with pathogenic organism for plants should be expected.

The most important pathogens found in soils, which pose significant threat to the forest tree saplings are above all the fungi of the following genera: *Phytophthora*, *Pythium*, *Fusarium* and *Cylindrocarpon*. Constraining their spread is extremely difficult due to high adaptability, large host scope and the presence of various infectious and endospore forms. The accumulation of pathogens in the soil niche in favourable weather conditions results in mass withering of saplings. For forest nurseries and cultivations, the existence of powdery mildew and downy mildew, and pine *needlecast* disease caused by *Lophodermium pinastri* and needle blight of larch caused by *Meria laricis* is unfavourable and may contribute to the decay of young saplings and the decrease of the value of plant material. Unfortunately, also with reference to those diseases, the choice of active substances of fungicides is limited.

3. Dithiocarbamates

The forest nurseries use above all preparations containing dithiocarbamates (Fig. 1), interacting with the fungal cells through disturbing various metabolic processes, the result of which is the inhibition of spore germination (Wong and Wilcox 2001).

Mankozeb belonging to dithiocarbamates in itself does not exhibit fungicidal activity but may be effective as the pre-fungicide, because when in contact with water it is decomposed, releasing sulfide ethylene bis-isothiocyanate (EBIS). This compound is then UV converted to ethylene bis-isothiocyanate (EIB). Both compounds are deemed as toxic and interact with the sulfhydryl groups of enzymes. Those reactions disturb basic enzymatic processes and lead to the death of cells. It is also believed that those compounds interfere with six various biochemical processes taking place in the cytoplasm and mitochondria of fungal cells (Gulliano et al. 2010).

Dithiocarbamates mechanism of action is multipoint, which causes that it is unusually advantageous from the practical point of view since it significantly reduces the possibility of the resistance of fungi conditioned upon the mutation of single genes. In such a case only the so called

polygene resistance may occur, caused by mutations in several genes, which may in turn trigger the mechanisms of detoxification of the active substance and lead to the occurrence of cross resistance (Gulliano et al. 2010).

The protective effect of that group of preparations is not however perfect, since they are contact substances and their effect is short-term. Despite the fact that preparations belonging to that group gained worldwide commercial success, due to the large scope of effectiveness in combating numerous groups of pathogens (*Ascomycetes*, *Oomycetes*, *Basidiomycetes* and *Deuteromycetes*), their effectiveness is high, however, only during early stages of infection.

4. Carboxylic acid amides (CAA)

The preparations belonging to the groups of CAA (carboxylic acid amides), to which dimethomorph belongs, were used for the first time at the beginning of the eighties.

The chemicals are effective in combat against *Oomycetes* from the family of *Peronosporaceae* (such as *Plasmopara viticola* and *Bremia lactuceae*) as well as *Pythiaceae* (genus *Phytophthora*, but not *Pythium*) (Gisi 2007). Their activity consists in disturbing the phospholipid biosynthesis in fungal cells, which has an adverse influence on the process of the creation of the cell wall. Those preparations inhibit also all processes related to the asexual reproduction of fungi but do not influence the development and mobility of zoospores (Wang et al. 2009).

Studying the activity of dimethomorph in laboratory conditions, it was found that the strains of *Phytophthora capsici* were moderately resistant (Young, et al. 2001). In Russia, the resistant strains *Phytophthora infestans* (related to the most important late blight pathogens found in Poland) sprang into existence due to frequent treatment application with the use of dimethomorph as the active substance (Dereviagina et al. 1999). The Internet website (www.frac.info) belonging to the organization FRAC (Fungicide Resistance Action Committee), associating researchers from all over the world, dealing with the phenomenon of fungi resistance to fungicides, revealed the information that the strains *P. infestans* are sensitive to the CAA preparations which is crucial for the forest officers and forest nurserymen (Moore, et al. 2008).

However, another species of fungi *Plasmopara viticola* proved to be completely resistant to any active substances from the CAA group (Moore, et al. 2008). This case is interesting because the resistance was broken due to the frequent repetition of protection treatment, which creates a real possibility of observing such a phenomenon in the case of other fungi. The resistance to CAA identified in laboratory conditions in the case of *P. viticola* is inherited recessively (Blum, et al. 2010), while in the case of *P. capsici* it is conditioned by two dominant genes (Meng, et al. 2011). The most

recent research carried out by Chen, et al. (2012) allowed for the identification, within the genome *P. melonis*, of the mutation related to the resistance to CAA preparations within the *CesA3* gene encoding a polypeptide made up of 1139 amino acids with a molecular mass of 126.5 kDa. The comparison of the amino acid sequences of isolates sensitive and resistant to CAA, proved the creation of mutation at the codon 1109 resulting in the conversion of amino acids: valine to leucine (Chen, et al. 2012). According to the authors the resistance to CAA of the *P. melonis* isolates may be controlled by the recessive gene(s), however, the confirmation of that thesis requires further genetic experiments (Chen, et al. 2012).

To sum up, the risk of increased resistance to active substances from the CAA group of *Oomycetes* on a large scale should be deemed as minute and may be additionally decreased by ceasing the repetition of protection treatment in short periods of time.

5. Carbamates

Carbamates are designated for combating *Pythium*, *Phytophthora*, *Aphanomyces* and some fungi from the *Fusarium* genus, i.e. pathogens causing wilt diseases of many plant species (Cohen and Coffey 1986, Englander et al. 1980, Rapp and Richter 1983).

The mechanism of propamocarb activity (Fig. 1), active substance from that group, consists in the disturbance of permeability of cell membranes of young mycelium, however, in the case of older mycelium or germinating spores in sporangium, its activity is not very effective (Papavizas, et al. 1978). Therefore propamocarb activity on plants strongly infested with *P. infestans* is invisible (Samoucha and Cohen 1990). The research results by Hu and Hong (2007) indicated that the preparation should be applied preventively in forest nursery before the infection of seedlings with zoospores. Propamocarb may slow down the disease process by inhibiting the production of spores (sporangia) and reducing zoospores activity. However, after the infection, the effect of the fungicide is lost because the treatment at this stage is not recommended, at the same time the risk that *Phytophthora* will get resistant increases. Prokamokarb does not have adverse effect on beneficial to plants microorganisms, which was proven with reference to the *Trichoderma* and mycorrhizal fungi (May and Kimati 2000, Wilde 1990). Therefore, the preparation should be used in the programmes of integrated protection against fungal diseases in forest nursery.

6. Phosphonates

Systematic preparations from the group of phosphonates (fosetyl aluminum and potassium salts of phosphoric acid)

(Fig. 1) belong to the most important chemicals designed for the reduction of diseases caused by fungi-forms *Oomycetes*, especially from the genus *Phytophthora*. Phosphonates are widely used in agriculture, horticulture, forestry and also in natural environment (Guest and Grant, 1991, Daniel et al. 2005). They are used in Africa, Asia, Australia, Europe and North America to protect rare and endangered species of plants and to protect crop. Those compounds in the form of water solution spray (along with the wetting agent) are also used in trees protection, or they are directly injected to the trunks, where they move in the plant tissues through phosphate pathway (Guest and Grant, 1991). Phosphonates are not metabolized by the plant, which results in the fact that they stay in the tissues for a considerable period of time (6–8 years), which is closely related to the species of the plant, its growth speed and the speed of losing leaves (Hardy et al. 2001). Those compounds have two different mechanisms of activity towards pathogen, depending on the amount of the active substance (Smillie, et al. 1989). High concentrations inhibit growth and sporulation of pathogens (Wilkinson, et al. 2001, Garbelotto et al. 2009); while low concentrations may indirectly stimulate the defence response of plants. Taking into consideration the fact that the concentrations of phosphonates in plant tissues rarely reach concentrations that had fungistatic qualities *in vitro*, stimulation of defence mechanisms seems to be of greater importance.

It was observed for a long time that the phosphonates action may be different as regards various plants grown by farmers, and one of the hypotheses says that the compounds above all stimulate defence mechanisms of an organism (Grant, et al. 1990, Daniel and Guest 2005). Fosetyl-Al is described in reference books as the elicitor of plant defence mechanism and its action results in the synthesis of phenol compounds in leaves. Dercks and Creasy (1989) provide the information that the activity of the compound in combating *P. viticola* was high, both in the pre-infection phase and after the infection. Comparing the activity of fosetyl-Al in the control of downy mildew on the grapevine varieties differing in resistance proved that the plant defence response was directly related to the ability of the plant to synthesize phytoalexins. The most resistant grapevine variety also strongly accumulated resveratrol in its tissues, which exerted very strong pressure on the fungus. The research by Dercks and Crease (1989) also showed a strong correlation between the plant resistance and the level of accumulation of resveratrol.

There is also a proof to the fact that the defence mechanisms of plants may be stimulated by the changes in pathogen metabolism. Research by Grant, et al. (1990) showed that low concentration of phosphonates may modify the metabolism of *Phytophthora* species without visible influence on the growth of mycelium. Guest and Grant (1991) hypothesized that the

preparations with phosphonates may exert influence on the pathogen by disturbing the synthesis of its suppressors, i.e. compounds ‘tricking’ the defence mechanism of the plant.

Very interesting, from the practical point of view, was the research carried out by Pilbeam, et al. (2011), which proved various reactions to infection triggered by *P. cinnamomi* when using phosphonates on resistant and susceptible line of eucalyptus. Phosphonates caused various histopathological responses in the tissues of eucalyptuses. Stimulation of mitosis, the creation callus and synthesis of lignin took place in resistant lines, while sensitive lines increased the production of lignin and suberin (Pilbeam, et al. 2011).

The risk of resistance of pathogenic *Oomyces* to preparations containing phosphonates due to its very intricate, non-specific mechanism of protective interaction is deemed as slight, however there are reports about diminished sensitivity of isolates *P. cinnamomi* (Dobrowolski et al. 2008) and *Bremia lactucae* (Brown et al. 2004).

7. Morpholines

Spiroksamina (Fig. 1) belongs to the morpholine preparations – an important group of fungicides having a large scope of usage directed towards powdery mildew, rust and scab (Pommer 1995). It exhibits the same way of the interaction with fungal cells and other active substances in the group (fenpropimorph, fenpropidine, tridemorph), site-specific inhibiting sterol biosynthesis (SBI class II) (Leroux et al. 1999). Sterols are a fundamental component of all cell membranes of eukaryotic organisms, and their presence is necessary for normal development (Joffrion and Cushion 2010). Sterols in fungi cells perform several functions: they are responsible for the fluidity and permeability of the cell membranes (Bard et al. 1978, Lees et al. 1979) and they regulate enzymes related to them (Cobon and Haslam 1973). Morpholines affect to varying degrees two specific points in the sterol biosynthesis pathway, inhibiting the metabolism dimethylergostatrienol to dimethylergostadienol by blocking $\Delta 14$ -reductase and fecosterol to episterol, by disrupting to the operation $\Delta^{8,7}$ – isomerase (Kerkenaar 1995).

The use of spiroxamine is recommended primarily to control of powdery mildew in different plant species, and in attempts to use it in the fight against other pathogenic fungi showed low efficiency of the active substance (Debieu et al. 2000).

There are many references in scientific books to the decrease of sensitivity of pathogenic fungi to fungicides containing morpholines: *Erysiphe graminis* (Felsenstein et al. 1994, Napier et al. 2000), *Microdochium nivale* (Debieu et al. 2000), *Botrytis cinerea* (Leroux et al. 1999), *Nectria haematococca* (Lasseron-De Felandre et al. 1999). Additionally due to the possibility of cross resistance in 2005, FRAC organization assessed the

risk of resistance to spiroxamine from low to moderate (www.frac.info). The problem of resistance to the preparations of the SBI group concerned the research of Zhu et al. (2008) which confirmed cross resistance to SBI in *Pseudoperonospora cubensis* (*Oomyces*). The resistant mutant strains were resistant to both flumorph and dimethomorph (various fungicides), and the results clearly indicate that this is the same mechanism of resistance, therefore the risk of resistance in this case should be considered as medium (Zhu et al. 2008).

Using the SBI preparations in forest nursery may carry some threats. In container nurseries, where the major problem is the occurrence of powdery mildew, morpholine substances can seep into nuggets of the substrate, which may lead to less efficient use of mycorrhizal vaccines. Morpholines used in laboratory conditions already in low dosages adversely affected the growth of slime mould and sporulation of the mycorrhizal fungus *Glomus intraradices*, which was related to the disturbance of sterols metabolism (Campagnac et al. 2009). Previous research carried out by the team under Campagnac (Campagnac et al. 2008) concerning morpholine preparations (fenpropimorph and fenhexamid), also showed a direct effect of the mentioned active substances on the degree of the mycorrhiza of roots by *Glomus intraradices*. Analyses conducted by researchers showed that the first of these preparations reduced this process dramatically (Campagnac et al. 2008). The use of fenhexamid also adversely affected another mycorrhizal species *Glomus larum* (Cardenas-Flores et al. 2011). The authors of the research found that high levels of concentration and the frequent repetition of treatments with the use of SBI fungicides has a negative impact on mycorrhizal fungi in soil (Cardenas-Flores et al. 2011).

The latest test results obtained by Kuc and Aleksandrowicz-Trzciska (2012) do not confirm the negative impact of fenhexamid on the development of ectomycorrhizal symbiosis. The authors report that the preparation Falcon 460 EC did not limit the mycorrhiza of oak seedlings with fungus *Hebeloma crustuliniforme*, on the contrary, the results indicate the possibility of stimulation of mycorrhiza formed spontaneously.

8. DMI preparations (triazoles)

Fungicides belonging to this group comprise about 30 active substances with high efficiency against a number of fungal pathogens. DMI preparations are used in reducing powdery mildew and fungi genera of rust and *Ustilaginaceae*, grey and white mould and other fungal pathogens that cause leaf spots.

Studies showed that triazole fungicides penetrate through the roots, stems and leaves, are absorbed in the xylem and moved acropetally in plants, and are not moved by phloem to roots, and therefore the mechanism of action of these substances should be defined as locally systemic (Edgington 1981).

Those compounds block the biosynthesis of sterols in fungal cells (Yang et al. 2011). The mechanism is based on the inhibition of 14- α sterol demethylase dependant on cytochrome P-450 at position 14 of lanosterol and to a lesser extent, on the inhibition of Δ^{24} -sterol methyltransferase (Elliott 1999). Most of the DMI fungicides inhibit cytochrome by attaching to the cysteine pocket in the active site of the enzyme. As the reference books indicate the field resistance to triazoles was found after about 10 years of use, in populations of powdery mildew and scab (Ma and Michailides 2005, McGrath 2001). Further research on fungal resistance to these fungicides helped to identify the forms of reduced sensitivity in populations: *Fusarium asiaticum* (Yin et al. 2009), *Fusarium asiaticum* (Yin et al. 2009), *F. solani* (Kalamarakis et al. 1991), *Microdochium nivale* (Cristani and Gambogi 1993). Due to spot interaction mechanism on the pathogen and a large variety of preparations of similar mechanism of action, the risk of obtaining resistance to the assessed preparations is medium with high probability of occurrence of cross resistance (www.frac.info).

Reference books say that DMI resistance mechanisms observed in populations of fungi include: mutation of the enzyme- 14 α -demethylase (CYP51) which leads to a reduction in the affinity of DMI with target protein (Dely et al. 1997), overexpression or increase of the number *CYP51* copies gene, results in an increased amount of the target enzyme (Hamamoto et al 2000), overexpression of transport proteins ATP-binding cassette (ABC) (efflux pumps) involved in transport of sugars, amino acids, proteins, peptides and metal ions (Zwiers et al. 2002), and unidentified resistance mechanisms to DMI (Mellado et al. 2001, Wood et al. 2001).

DMI preparations outside of the fungicide properties exhibit indirect action on bacterial cells, although they do not contain sterols. The research work showed that triticonazole stimulates proliferation of bacteria in the soil, while fenpropimorph and propiconazole completely inhibit the activity of the soil bacteria (Milenkovski et al. 2010).

Triazole compounds due to the mechanism of modifying the sterol pathway of different organisms also exhibit interaction similar to the effect of growth regulators on plants (Barnes and Kelley 1992 Coolbaugh et al. 1982). Some triazole formulations are used as plant growth retardants (growth inhibitors) (Rademacher 2000). The DMI action in this respect consists in the inhibition of the biosynthesis of gibberellins, which leads to the reduction of the elongation growth of roots and shoots (Görtz et al. 2008).

It turns out that despite the broad activity of DMI preparations influencing both the fungi, bacteria and plants, the triazole compounds do not indicate fungistatic interaction with *Oomycete*. Responsible for this is the large phylogenetic distance separating relevant fungi and *Oomycete*, which translates directly to the varying effectiveness of plant

protection chemicals in reduced occurrence of these distinct groups of organisms. *Phytophthora* cells and other species belonging to the *Oomycetes* do not contain ergosterol, and their genomes are not functional *CYP51* genes (Tyler et al. 2006). These organisms, however, have the ability to produce the squalene and to transform exogenous sterols, and therefore they can be regarded as auxotrophic organisms towards obtaining sterols (Marshall et al. 2001). *Oomycota* charge precursors for sterols of the host plant during infection in which a large family of extracellular proteins structurally related to the transfer of lipids act as agents. These proteins are involved in plant-pathogen relationships, and their presence results in activation of the pathogenesis of hypersensitivity reactions (Blein et al. 2002).

The triazoles are used in the nurseries and forest cultivation for intervention in the control of oak powdery mildew (*Erysiphe alphitoides*), pine needlecast disease (*Lophodermium pinastri*, *L. seditosum*) and needle blight of larch (*Meria laricis*) (Fig. 1).

There are also examples of the use of DMI preparation in older stands. In the U.S., propiconazole is used to control oak wilt *Chalara quercina*. The effectiveness of that active substance in this dangerous disease of oak trees was documented in research by Eggers et al. (2005) as well as Peacock and Fulbright (2007). Carrying out the treatment procedure consists in the introduction of a fungicide through macro- or micro-injection into the part of wood, involved in the transport of water (Wilson and Lester 2002). Implementation of microinjection involves the introduction of small volumes of concentrated active substance by micro-injectors through a drilled hole in wood. The number of microinjection depends on the diameter of the tree, and their distribution is even. The mechanism of the collection of fungicide through tree tissues is primarily passive, it may also be forced by injectors (Haugen and Stennes 1999). The microinjection procedure introduces concentrated active substances (4-8 ml) in a larger volume of water. Studies by Haugen and Stennes (1999) showed that large volumes of dilute fungicides are better distributed in the tissues of the trees and thus are more effective. The protective effect of prothioconazole in the decline of oak lasts for two years from the application of the product (Eggers et al. 2005), but its application is not effective when the roots are infected (Wilson and Lester 2002).

9. Aminopyrimidines

Aminopyrimidines constitute a group of systemic active substances effective against powdery mildews (*Erysiphales*), to a lesser extent used in the control of rust and the smut. Bupyrimate belongs to this group of fungicides whose mechanism of action consists in inhibiting the synthesis of nucleic acids (Hollomon 1979), by reducing the activity of adenosine deami-

nase (ADA), the enzyme involved in purine metabolism, which catalyzes the deamination of adenosine to inosine (Hollomon and Chamberlain 1981). It was proven that aminopyrimidine fungicides should be used in the early stages of infection, because they inhibit the appressoria and haustoria of powdery mildew, the use of this group of active ingredients may also be useful in the later stages of development, because they inhibit spore germination (Hollomon and Schmidt 1987).

The first aminopyrimidine preparations were introduced in about 1968, they were initially very effective in mitigating powdery mildew of cucumber (*Sphaerotheca fuliginea*), but after about two years due to the frequent repetition of protective measures, in the populations of *S. fuliginea*, the resistance to these active substances was observed (Brent 1982).

The occurrence of resistant forms to the aminopyrimidines was also confirmed in the population of powdery mildew of barley (*Erysiphe graminis* f.sp. *hordei*) (Hollomon 1979). So far, the mechanism of fungal resistance to this group of active substances has remained unclear. Brent and Hollomon (2007) indicate that resistance of powdery mildew to aminopyrimidines may be less rapid, but should be defined as a continuous and progressive. According to the authors the pathogen population may return to the state of sensitivity to the preparation, if the protective treatments are performed less intensively with simultaneous use of other active substances (Brent and Hollomon 2007).

In conclusion, it should be noted that recommended for the control of powdery mildew and pine rash aminopyrimidine and triazole active substances, aimed at helping to reduce the incidence of these types of diseases, have extremely narrow mechanism of action to the fungus. Of course, the intensification of protective treatment in the situation when other preferred active ingredients are missing, may result in the occurrence of resistance to a larger scale. Additionally, reducing the occurrence of powdery mildew is extremely difficult because of the huge propagation potential (heavy sporulation), rapid life cycle and broad temperature and humidity preferences of this group of organisms. Inhibition of disease symptoms is achieved by frequent repetition of protective measures, while the limited access to active substances results in the need of a search for alternative methods to combat these pathogens (Pap et al. 2012).

10. Inorganic preparations (copper oxychloride)

Inorganic compounds are the oldest group of active substances used in plant disease control. The first reports on the use of organic copper date back to 1761 when copper sulfate (CuSO_4) was used in very high concentrations to protect wheat against the smut. Works on the use of copper in the control of plant diseases were continued by Prevost (1807),

who reported the inhibition of germination of spores of the smut. Further studies on the use of copper were carried out by a botanist, professor of Bordeaux Millardet who around the year of 1885 adopted 'Bordeaux mixture' ($3 \text{Cu}(\text{OH})_2 \cdot \text{CuSO}_4 \cdot \text{CaSO}_4$) to protect the vines against *Plasmopara viticola*. In subsequent years, copper preparations were also used in the potato late blight control. The greatest progress in the study of copper-preparations fell on years 1925–1935, when the active substances of this group were first patented.

Currently in plant protection, copper ions (Cu^{2+}) are used in the form of copper sulfate, copper oxide, copper naphthenate, copper oxychloride, copper (II) 8-hydroxyquinolate. Copper preparations are used to control: *Plasmopara viticola*, *Phytophthora infestans* and other fungi that cause leaf spots and in forestry to reduce the incidence of pine *needlecast* disease (Fig. 1).

The mechanism of interaction of copper on the fungal cells is complex. Copper ions have an affinity to various chemical groups found in fungal cells and react with thiol groups, resulting in non-specific denaturation of proteins and enzymes. They form stable complexes with the coenzymes and other biologically active compounds, which leads to a reduction in metabolic activity of fungi. Copper preparations limit the respiratory processes in the cells of fungi by inhibiting the formation of acetyl-CoA, interrupt process in the respiratory chain of phosphorylation and the same time inhibit the formation of ATP.

The effect of copper preparations is not limited to pathogenic fungi. The research with the use of copper oxychloride proved their negative impact on the population of mycorrhizal fungi *Glomus* spp. and *Arachis hypogea* L. (Sreenivasa and Bagyaraj 1989) as well as the neutral impact on species *Glomus fasciculatum* i *Agrostis palustris* L. (Rhodes and Larser 1981). The obtained different results of studies resulted from different impact of active substance on mycorrhizal fungi in different plant species (Rhodes and Larser 1981, Sreenivasa and Bagyaraj 1989).

Other research proved the decrease in the activity of soil microorganisms and the inhibiting impact of the copper preparations on ectomycorrhizal fungi in environmental tests on pine saplings (Manninen and et al. 1998). Further studies on copper oxychloride showed further reductions in the growth of mycorrhizal fungi: *Cantharellus cibarius*, *Corticium bicolor*, *Paxillus involutus* and *Suillus* (Laatikainen and Heinonen-Tanski 2002).

The several data also suggest the possibility of adverse effects of copper preparations on plants. Gibson (1958) showed that copper compounds used on acidic soils (copper oxychloride, copper oxide) had phytotoxic effect on pine species *Pinus radiata* and *P. patula*. Adverse interaction consisted in premature demise of the root apex, and eventually the death of plants; this mechanism has been still poorly understood (Orbovic et al. 2007).

Reference books provide the information that copper ions are effective against some fungi causing wood decay. From the study carried out by Chen (2010), it follows that defence

mechanism of a tree against fungi consists in the binding of copper and polysaccharides and lignins in the wood cell walls. Very good for this process is the migration of Cu^{2+} in wood (Choi et al. 2001). Copper preparations show lower effectiveness against white and brown rot of a tree, caused by fungi from the families *Antrodia* and *Serpula*, which create non-toxic crystals of copper oxalate through binding the copper ions (Hastrup et al. 2005). Improved efficiency of the organic copper preparations in reducing wood damaging fungi may be obtained by the combined use of the quaternary ammonium salts (QAC-active substances destroying bacteria, less effective for fungi), which in combination with copper ions produce a synergistic effect (Härtner and Barth, 1996).

11. Derivatives of aromatic hydrocarbons

Derivatives of aromatic hydrocarbons belong to the group of fungicides having a benzene ring in the molecule, as well as having a different activity against fungi. Chlorothalonil (TPN, -2, 4, 5, 6 tetrachloroisofthalonitrile) – was recorded for the first time as a fungicide in 1966 and was recommended for wide use in agriculture, horticulture and forestry all over the world (Wang et al. 2011). The preparation has a very broad spectrum of activity, mainly as a fungicide, and in particular as a powdery mildew control agent. It has also properties against bacteria, algae and insects. The exact mechanism of its action has not been known, but it can be characterized as an inactivation of the glutathione-related enzymes involved in the respiratory processes of fungal cells (Arvanites, Boerth 2001). Fungicide Resistance the Assessment of Risk (FRAC, www.frac.info) characterizes the preparation as a means of multipoint contact mechanism of acting against the pathogen, that causes low risk of occurrence the resistance in fungi populations. The studies also showed phytotoxic effect of the formulation compared to seedlings and cuttings of forest tree nurseries (James, Woo 1984). In the experiments, the phytotoxic effect of the preparation for tree seedlings in nurseries was also showed (James, Woo 1984). Adverse effect of the preparation in relation to tree seedlings was also confirmed by Laatikainen (2006), proving that chlorothalonil applied in the container nurseries reduced the growth of pine seedlings and delayed their development, and thus their hardening. The effect of the preparation was maintained for a long time; after two years, changes in the content of nitrogen and free amino acids in the pine seedlings were evident (Laatikainen 2006). It was also showed that chlorothalonil has highly toxic effect on soil fungi populations (Sigler, Turco, 2002), whereas in pure cultures it appeared to be highly toxic to ectomycorrhizal fungi (Laatikainen and Heinonen-Tanski, 2002). The above observations, however, were not confirmed during the experiments conducted by Aleksandrowicz-Trzcńska (2008),

in which chlorothalonil did not limit the development of mycorrhizas in the pine regeneration. TPN preparation is also highly toxic to fish, aquatic invertebrates and birds (Caux et al., 1996), as well as harmful to human being (Draper et al., 2003).

12. Pyridinecarboxamide inhibitors of succinate dehydrogenase (SDHI)

Introduction to use of first generation SDHI fungicides (inhibitors of mitochondrial complex II: carboxin and oxy-carboxin) dates back to the late sixties of XX century. These were compounds which inhibit respiratory processes, effective in combating diseases caused mainly by Basidiomycetes (Zhang et al. 2009). The target site of their action is the succinate dehydrogenase complex (SDH) in respiratory chain (complex II) in binding site of ubiquinone (SQR), due to the distortion of the electron transport, mitochondrial respiration is inhibited (Yin et al. 2011). The second generation inhibitors of succinate dehydrogenase which comprise boscalid (registered in 2003), are the preparations with a broad spectrum of activity against pathogenic fungi to many crop species (Avenot, Michailides 2010). The preparation is bound in a waxy layer of cuticle, as well as moves in the tissue at the inner side of the leaves. According to the EFSA report, boscalid is the fourth most common pesticide present in food (EFSA 2013). Undoubtedly, the popularity of the product translates into a high risk of genetically conditioned resistance. FRAC organization assesses the risk of resistance to SDHI preparations from medium to high (www.frac.info), therefore for basic FRAC research, an obligation to monitor the plant resistance to pathogenic fungi on SDHI preparations was introduced.

In accordance with the recommendations of FRAC, due to the presence of cross-resistance within the SDHI group, the preparations should be used prophylactically if the risk of the disease is high. They should not be used in emergency, particularly when the pathogen population is large (McKay et al. 2011). These preparations should be used up to 2 times during the growing season best in combination with another active ingredient, or alternatively with substances from other groups that do not exhibit cross-resistance (McKay et al. 2011). SDHI preparations do not exhibit cross resistance with other classes of fungicides: strobilurin, anilinopyrimidines and benzimidazole, therefore, in order to eliminate any of resistant strains, they should be used in combination with other active substances. (Stammler et al. 2007).

The recent studies on the action of boscalid on soil-forming processes showed that it is a persistent compound with little mobility having a negative impact on conversion of phosphorus and carbon as well as respiratory processes in soils (Xiong et al. 2014).

13. Strobilurin fungicides

Strobilurins are one of the most important groups of fungicides with the wide antimycotic spectrum, effective against Ascomycetes, Basidiomycetes and Oomycetes. For the first time, strobilurin (azoxystrobin) was obtained in 1977 from the mycelium of *Strobilurus tenacellus*, a species of decaying wood (Schramm et al., 1978). So far, about 20 strobilurin analogs have been isolated from basidiomycetes such as *Oudemansia iellamucida*, *Pterula* sp., *Xerula longipes*, *Mycena crocota*, *Favolaschia calocera* and ascomycetes, for example *Bolinea lutea* (Malita, 2008). Fungicides belonging to this group are highly prized for strong activity, low toxicity to mammalian cells and environmentally sound (Zhang et al. 2012).

Strobilurins act by inhibiting mitochondrial respiration in fungi cells. They interfere with the energy processes by blocking electron transfer through mitochondrial membrane, between cytochrome b and cytochrome c1 at the site of oxidation of quinol (Qo), thereby preventing the formation of ATP, which interrupts the energy cycle of the pathogen (Ammermann et al., 2000).

Pyraclostrobin is a fungicide from the group of strobilurins, which acts as a brake on the growth of hyphae and sporulation (Ammermann et al. 2000). In plants it moves in vascular system and translaminary (moves from one side of a leaf to the other). Strobilurins also regulate plant growth and development. It was demonstrated that pyraclostrobin applied to healthy plants caused an increase in the biomass due to the increased nitrogen uptake (Köehle et al. 2003). It was also shown that QoI fungicides inhibit respiration in plants (Glaab and Kaiser 1999), and produce other physiological changes like modification of plant hormones leading to the delay of leaf senescence (Ruske et al. 2003). Preparations of this group reduce stomatal conductance and reduce water consumption, leading to an increase in the photosynthetic activity of the plants (Grossman et al. 1999), and they increase the activity of antioxidant enzymes (Wu, Tiedemann 2002). Highly specific mechanism of strobilurins action on fungal cells and their frequent application promote rapid immunization of pathogens to this group of fungicides. The resistance mechanism is noted throughout the world in populations of pathogens of crops, fruits, vegetables, ornamental plants and grasses (Fernández-Ortuño et al. 2008), and the risk of its occurrence is rated very high. The FRAC monographs revealed the information that field resistance to strobilurins was identified in populations of more than 30 species of fungi pathogenic to plants (www.frac.info). It was demonstrated that for the resistance to the QoI preparations are responsible point mutations (single nucleotide polymorphism) in the cytochrome b gene sequence: leading to the conversion of three amino acids: glycine to alanine change at position 143 (G143A), the conversion of phenylalanine to leucine at position 129 (F129L) and exchange of glycine for arginine at position 137 (G137R) (Kim et al. 2003,

Sierotzki et al. 2006). The effect of these mutations is an alternative respiration and increased activity of the transport protein in ATP-binding cassette (ABC) membrane-associated cellular responsible for the mechanism of naturalization of fungicides (Fernández-Ortuño et al. 2008). Some FRAC publications include the information indicating how to use the QoI fungicides to minimize the risk of resistance of pathogenic fungi. Due to the high efficiency in inhibiting spore germination, these preparations should be used prophylactically. They can be used alone (in blocks with other fungicides), or alternatively with preparations from different group of cross resistance. QoI preparations can also be used in admixture with other active compounds, but only those which have a different mechanism of action on the cells of the pathogen, which broadens the spectrum of protective treatment and improves its efficiency. Active substances to be combined with strobilurins should be chosen in such a way that their efficiency on the target population of pathogens in the separate application would be high (www.frac.info). Currently, much effort has been focused on modifying the structure of the strobilurin in order to obtain new compounds with antifungal properties that are effective on the populations of resistant pathogens. Research shows that an effective way to acquire new strobilurins are modifications of the side chain (Li et al. 2006).

14. Benzimidazoles (MBC)

Benzimidazoles are a group of systemic fungicides developed in 1968, showing high activity against vascular and soil borne pathogens. It is a group of fungicidal preparations, the benzimidazoles contain in their chemical structure both benzene and imidazole rings. In fungal cell they inhibit cell multiplication (Yang et al. 2011). MBC fungicides bind β -tubulin in microtubules and inhibit their proliferation (Koo et al. 2009), which disrupts the mitotic spindle assembly of mikotubul, cellular transport and the formation and function of the cytoskeleton (Rathinasamy and Panda, 2006). Thiophanate-methyl belonging to the group of MBC fungicides is a systemic preparation with broad spectrum of antifungal properties. It is used in the protection of cereals, gardening, horticulture and forestry, as well as in the protection of timber (Cycoń et al. 2011).

The first signs of fungal resistance to MBC group of preparations were recorded in 1973, and now the resistance has been identified in the case of many phytopathogens species. In most cases, it was associated with the presence of point mutations in the β -tubulin gene, which led to amino acid changes in the binding site of the benzimidazole. Literature data show that the resistance of many plant pathogenic fungi to MBC is often due to mutations in codon 6, 50, 167, 198, 200 and 240 of β -tubulin (Ma, Michailides 2005). Resistance to MBC has characteristics of cross resistance to all preparations belonging to this group (www.frac.info).

Table 1. Fungicides used in forest nurseries (Głowacka et al. 2013)

No.	Group of fungicides	Active substance	Preparation	Introduction date of active substance	Scope of activity	Mode of action	Use recommendation in forest nurseries	Confirmed resistance to active substance		
1	Carboxylic acid amides	dimetomorph	Acrobat MZ	around 1980	deep-seated	inhibition of cellulose synthesis in Oomycetes	seedling blight	resistance of <i>Phytophthora capsici</i> in laboratory conditions (Young et al. 2001); <i>Plasmopara viticola</i> in the environment		
		69 WG								
		mankozeb	around 1960						contact	
2	Dithiocarbamates	mankozeb	Penncozeb 80 WP	around 1960	contact	disturbance of metabolic processes, inhibition of spore germination	seedling blight / phytophthora disease / shed of pine needles	low risk due to multi-point mechanism of action, <i>Botrytis cinerea</i> – resistance under laboratory conditions (Barak et Edgington 1984)		
		thiram	75 DS/WS						around 1960	contact
		thiram	Thiram Granuflo 8 WG						around 1960	contact
		methiram	Polyram 70 WG						around 1960	contact
3	Carbamates	promao carb	Previcur Energy 840 SL	1978	systemic	disturbance of permeability of cell membranes	seedling blight – pine	<i>Pythium</i> spp. in the environment (Moorman et al. 2002, 2004);		
4	Phosphonics	fosetyl aluminum		1977	systemic	direct fungistatic-high doses, indirect elicitors or suppressors		<i>Phytophthora citrophthora</i> under laboratory conditions (Angeles Diaz Borrás et Vila Aguilar 1988)		

No.	Group of fungicides	Active substance	Preparation	Introduction date of active substance	Scope of activity	Mode of action	Use recommendation in forest nurseries	Confirmed resistance to active substance
5	Ketamines (morpholines)	spiroxamine		1999	sterol biosynthesis inhibitors of class II			<i>Erysiphe graminis tritici</i> (Napier et al. 2000); <i>Nectria haematococca</i> under laboratory conditions (Lasseron-De Felandre et al. 1999)
			Falcon 460 EC/ Sokót 460 EC				oak powdery mildew / pine needlecast	
6	DMI (triazole)	tebukonazole		1986	systemic	inhibitors of sterol dimethylation of class I		1* <i>Fusarium asiaticum</i> , <i>Fusarium graminearum</i> (Yin et al. 2009); <i>Fusarium solani</i> (Kalamarakis et al. 1991); <i>Microdochium (Fusarium) nivale</i> (Crisci et al. 1993)
			triadimenol		1973			
7	Pyrimidine	buprimate	Nimrod 250 EC	1976	systemic	deminases of adenosine (disruption of nucleic acid synthesis)	oak powdery mildew	<i>Erysiphe graminis</i> f.sp. <i>hordei</i> – in the environment (Hollomon 1979); <i>Sphaerotheca fuliginea</i> – in the environment (O'Brien et al. 1988)
8	Inorganic	copper oxychloride	Miedzian 50 WP	around 1925	contact	non-specific denaturation of proteins and enzymes	larch needlecast	unknown
9	Derivatives of aromatic hydrocarbons	chlorothal-nile	Gwarant 500 SC	1966	contact	inhibitors of respiratory processes	seedling blight / leaf spots disease / grey mould / pine needlecast	low risk due to multi-point mechanism of action

No.	Group of fungicides	Active substance	Preparation	Introduction date of active substance	Scope of activity	Mode of action	Use recommendation in forest nurseries	Confirmed resistance to active substance
10	Pyridinecarboxamides	boscalid	Signum 33 WG	2003	inhibitors of respiratory processes, inhibitors of cytochrome b of mitochondrial complex III	seedling blight / rust / grey mould / phytophthora / oak powdery mildew	<i>Alternaria alternata</i> (Avenot et Michalides 2007); <i>Cercospora cassicola</i> (Miyamoto et al. 2009); <i>Botrytis cinerea</i> (Yin et al. 2011); <i>Monilinia fructicola</i> (Chen et al. 2013)	
11	Strobilurins (QoI)	pyraclostrobin	Signum 33 WG	2001	inhibitors of respiratory processes, inhibitors of succinate dehydrogenase of mitochondrial complex II	30 species of pathogenic fungi		
12	Benzimidazoles (MBC)	thiophanate-methyl	Funaben Plus 03 PA	1970	inhibition of cell multiplication	diseases and damage of branches and trunks	many species	

Although there is no evidence of a direct negative impact of MBC preparations on the soil bacteria population, it was shown that the presence of MBC fungicides in the soil inhibits nitrification mediated by bacteria (Chen et al. 2001). However, the negative effect on mycorrhizae fungi was clearly demonstrated e.g. in the case of the arbuscular species - *Glomus mosseae* after the use of the half of the dose of the fungicide from the MBC group the inhibition of the growth of mycelium and spore formation was observed (Chiocchio et al. 2000).

15. Summing up

The use of fungicides is currently the most effective method of reducing of plant pathogenic organisms. However, the forest nursery production due to the limited size, is not an attractive market for fungicide manufacturers, which is expressed in a small number of preparations available in an organized forestry production. Undoubtedly, some antidote to cure this situation is the obligation to apply the principles of Integrated Pest Management (IPM), which applies to all branches of organized crop production. On the other hand, modern forest nurseries, due to the ever-increasing production, suffer from the presence of increasing number of diseases, resulting in financial losses. Therefore the use of alternative protection methods to improve the quality of phytosanitary nursery products (free of pests) as well as lobbying to introduce by producers modern fungicides with a broad spectrum of activity more often and faster to nursery production are essential. At the same time in order to preserve the principles of good plant protection practice, the duty of monitoring fungicide resistance development with the help of existing research units should be mandatory for all branches of plant production and thus minimize the risk of its occurrence.

Conflict of interests

The authors declare absence of potential conflict of interests.

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Author's contribution

A.O., T.O., A.P., J.N. – study conception; A.O.– manuscript preparation, T.O, J.N.– editing, proofreading; A.P. – data table preparation, data collecting.