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SCIENTIFIC SESSION III
MULTIFUNCTIONAL USE AND SUSTAINABLE MANAGEMENT
OF FOREST RECOURSES

PLENARY LECTURE
CONROLING STRATEGY OF THE PATHOGENS IN
FORESTRY NURSERY PRODUCTION

Snežana RAJKOVIĆ, Mara TABAKOVIĆ-TOŠIĆ,
Vesna GOLUBOVIĆ-ĆURGUZ¹

Abstract: *The forest seedling material production faces the major problem of the investigators of plant diseases, most notably the phytopathogenic fungi which causes the deseases of lodging of saplings. Their massive emergence is due to the fact that the production takes place in the open space where the sapling, during the period in which it is the most sensitive, is exposed to the numerous harmful abiotic factors, that are favourable to the development of pathogen, such as: Fusarium spp., Botrytis spp., Rhizoctonia spp. and Penicillium spp.*

The appropriate protection of saplings in the hothouses in the open space can be performed by applying the certain types of fungicides, specific effect of which is determined by the presence of the active substance.

Active ingradient which have fungicide effect and fall into the group of benomyl, ditiocarbamates, strbylirines, and triazol have the stisfactory results in the preliminary experimental investigations of the efficacy of the fungicides belonging to the aforementioned groups. Nevertheless, the current legal regulation forbids the circulation of pesticides which are not permitted to be used for elimination of certain pathogens on certain host plant. Theredore, if the preparation has not been registered for the use on the forest plant species, it cannot be used for protection. Due to the fact, there is a problem in Serbia as the producers of pesticides are not willing to register their products in forestry, since there is a small market demand for them.

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The strategy that we suggest refers to the selection of active substances and preparations which can be used in the forest hothouses. We also recommended the producers and beneficial owners of pesticides to investigate the effect of their products, in order to reister them in the category of small crops, in which the forest hothouses belong.

In this way, with the respect of the legislation, the control of pathogens which cause the lodging of saplings can be well-performed.

Key words: strategy, fungicides, pathogen causing lodging of saplings

INTRODUCTION

Diseases are important limiting factors in the production of forest seedlings within nurseries. Environmental conditions within nurseries are often ideal for the proliferation of disease-causing pathogens. Seedlings are usually grown in extensive monocultural systems either outdoors within fields (bare root) or within greenhouses (container). High moisture and nutrients supplied to nursery seedlings often promote proliferation of important pathogens.

The organisms inducing nursery diseases are unlike those that cause diseases of trees within forests. They are similar or the same as those pathogens that cause diseases of agricultural crops. Pathogens associated with nursery-grown seedlings are usually not problems once seedlings are planted in forest areas. Management procedures to reduce impact of diseases within forest nurseries are similar to those used to control agricultural pests. Therefore, many of the chemical and non-chemical control methods used in forest nurseries have been adopted from agricultural system that have similar pathogens.

OVERVIEW OF FOOREST NURSERY DISEASES CONTROL

I CULTURAL: diseases prevention is the main objective of cultural management of nursery diseases. Reduce inoculum in growing areas and avoid conditions which promote disease spread. Sanitary practices including greenhouse, containers, and seed.

II BIOLOGICAL: some commercially-available biocontrol materials have shown some promising results.

III CHEMICAL: use as a last resort. Fungicides often are specific to a particular group of fungi, as ase the application methods so a proper diagnosis is very important.

MAJOR DISEASES IN NURSERIES:

Fusarium spp. – seed rot, damping off, rood diseases, stem blight

Phytophthora spp. – root disease

Pythium spp. – root disease

Botrytis cinerea – foiar blight, storage mold

Cylindrocarpon destructans – root decay

Damping-off is the disease term used for fungal-caused mortality during those first critical few weeks from germination to just after seedling emergence. The soil-inhabiting fungi associated with damping-off are capable of causing rapid decay and mortality of seeds and germlings. These fungi are not host-specific. Disease and hosts Many species of fungi, often common soil saprophytes, are associated with damping-off and root rot. They become pathogenic when temperature, moisture, soil pH, and other conditions become favorable. In forest nurseries, *Pythium* and *Fusarium* species are the most common damping-off fungi. Others are *Rhizoctonia solani*, *Macrophomina phaseoli*, *Botrytis cinerea*, and *Phoma*, *Alternaria*, and *Phytophthora* species. In general, *Pythium* species cause problems early in the season when soils are cool and wet, while *Fusarium* species cause problems later when soils are warmer and moist to semi-dry. Exceptions occur, however, and any of these fungi can and do cause disease at any time during the growing season. Damping-off fungi occur naturally in nearly all crop and forest soils. They are found worldwide in temperate and tropical zones alike. No conifer or hardwood is known to be resistant to damping-off. Those species or seedlots that germinate quickly and grow fast may sustain less damage from damping-off than slow-emerging, slow-growing species. Still, it is safe to assume that all nursery-grown tree species are susceptible to damping-off fungi. Symptoms Damping-off is defined as the fungal invasion of the succulent tissue of germinants or seedlings that leads to decay and early death. Damping-off attacks seedlings both before emergence (preemergence damping-off) and after (postemergence damping-off) and, depending on conditions, usually occurs within 30 to 45 days after sowing. The only evidence of preemergence damping-off in nursery beds is that the germinating seedlings are sparse and patchy. This phase is difficult to detect, but may sometimes be diagnosed by digging up seeds that have not emerged and checking to see whether seeds or germinants are decayed or withered. Postemergence damping-off, which occurs in the cotyledon stage, causes seedlings to wither and collapse. When the succulent root-collar tissue or the roots are penetrated by the pathogen, the disease is referred to as soil-infection damping-off. When the fungal invasion occurs higher on the stem or cotyledons, it is called top-infection damping-off. The most obvious indicator of postemergence damping-off is the collapse of the seedling. It may be possible to tentatively identify, at least to genus, the fungus responsible. Stem tissues of seedlings infected by *Pythium* sometimes separate around the root collar, with the epidermis sloughing away from the inner xylem tissue as an open shirt collar falls away from the neck of the wearer. The trees then fall over. Seedlings infected by *Fusarium* undergo a softening of the root-collar tissue, and the trees fall over at the point of softening

without separation of the stem tissues. Seedlings with suspected *Fusarium* infection may be incubated over-night at room temperature in a moistened paper bag to produce a bloom of sickle-shaped macroconidia that can be easily identified under a microscope. In broadcast-sown beds, which are uncommon in Pacific Northwest nurseries, seedlings may die in irregular bull's-eye patches, with the centers containing mostly fallen trees and the borders containing trees with early symptoms. In drill-seeded beds, the mortality pattern usually runs along the rows for a distance, then abruptly stops.

Adjoining rows may be affected, showing a patchy effect. It is not unusual for forest pathologists to isolate one or more species of damping-off fungi from apparently healthy seedlings within the first few weeks of germination. The fungi may be found either on root surfaces or within internal tissues and may have come from infested seed or surrounding soil. These symptomless seedlings usually remain healthy as long as moisture stress is low and other growing conditions are optimum. Fungus biology Damping-off fungi are inhabitants of the soil. They can be spread by movement of soil on equipment or seedlings, by cultivation, or by water.

Infection occurs when seedling roots grow next to fungal inoculum, such as chlamydospores, sclerotia, or oospores. These structures then germinate and hyphae invade the seedling cells. Fungal invasion causes collapse and disintegration of cells and death of the seedling. The fungus may continue to develop in and utilize the killed tissue, often producing secondary inoculum, such as conidia, on the surface of the dead seedling. Mycelium, spores, or Damping-off may be confused with: Cutworm damage, Frost heaving, *Fusarium* hypocotyl rot, Heat damage Seedcorn maggot damage, other structures survive and over-winter in seedling tissue or other organic material in the soil. Viability of overwintering inoculum is dependent on a number of factors, including soil moisture and temperature. Loss potential Damping-off fungi can cause significant losses in forest nurseries. Losses may be large one year and minor the next. Mortality from preemergence damping-off can be estimated by calculating the difference between the number of seedlings and the number of seeds sown, after other factors, such as percent germination or bird depredation, are accounted for.

Postemergence damping-off is best determined by marking small plots and counting mortality every few days. Individuals can be marked with toothpicks monitor whether the disease is increasing, subsiding, or responding o treatment. Losses from preemer- gence damping-off often range from 15 to 40 percent of sown seed, while postemergence losses may be an additional 10 to 20 percent. Growers typically oversow to ensure a satisfactory crop. Damage may be heavy in seedling beds that previously contained transplants or other agricultural crops. In fact, new nursery sites developed from cleared forest soils tend to have fewer damping-off problems than those established on previous agricultural croplands. In

addition to the direct losses of bed stock, indirect losses may be reckoned in shortages of healthy seedlings for outplanting on forest sites.

MANAGEMENT

The best defense against diseases in forest nurseries are an effective and conscientious disease prevention program because pathogens can spread very quickly and cause extensive damage in relatively short time periods within nurseries. This includes knowledge of soilborne disease populations, a well-orchestrated pesticide program, and careful attention to environmental conditions in the nursery. The nursery environment is the strongest influence on the proliferation of damping-off fungi. Soil moisture, timing and amount of irrigation, air and soil temperatures, method and timing of sowing, depth of soil over seed, soil pH, combinations of soil fungi and nematodes, timing and type of nutrients applied, type of organic matter, type of cover crop, history and pattern of pesticide use, and many other factors affect the incidence and severity of damping-off. It is to the grower's advantage to bring the entire crop through its initial growth stages rapidly and evenly in order to narrow the damping-off infection "window." No single factor alone governs control of the disease, but good management will take the following factors into account:

Once disease symptoms appear, pathogen infection has usually been extensive and therapeutic treatments are often non-effective. Therefore, successful management of nursery diseases often involves reducing inoculum of potential pathogens in seedling production areas. Another major approach is to promote conditions which are non-conducive for pathogen infection are spread. Treating with chemical pesticides is often the last desirable and often last-implemented management approach, although sometimes this is the only way to prevent extensive losses.

Successful management of nursery diseases often involves reducing pathogen inoculum in seedling production areas.

SOIL MOISTURE AND DRAINAGE

Ideally, nurseries should be located on light, well-drained soils. Wet soil generally favors damping-off. Depth of irrigation is critical to young seedlings, especially during hot weather. It is important to irrigate deeply enough for water to reach seedling roots—a depth that increases steadily as the roots grow—but not so much that the soil is saturated. Too-shallow watering stresses tender roots in moisture-deficient lower soil layers, while creating a warm, wet upper soil layer that favors the buildup of damping-off fungi. Soil moisture and rooting depth should be monitored regularly during the growing season. Cool weather that prolongs germination, or hot weather that speeds it up, require particular attention to watering.

TIMING OF SOWINGS

Sowing when temperatures are warm enough to promote rapid, even germination tends to reduce problems with damping-off. Warm-weather sowing requires constant diligence in controlling irrigation.

SOIL pH

Damping-off fungi thrive in neutral to alkaline soils. A soil pH of between 5.2 and 5.7 (moderately acid) not only helps prevent damping-off problems but is ideal for growing Pacific Northwest conifer species. Aluminum sulfate drenches, sulfur (200 to 500 pounds/acre), and acid peat applications can be used to maintain the acid condition of the soil. When aluminum sulfate is used, beds should be kept moist to prevent burning of the roots. Irrigation water that is even slightly alkaline can, over a period of years, decrease soil acidity. The change usually occurs slowly because of the tremendous buffering ability of the soil. It can be reversed by acidifying the water with either sulfuric or phosphoric acid. The acidification process can be speeded up by adding sulfur to the soil and then maintaining the pH with acidified water.

SOIL MICROFLORA

No two nurseries are alike in their makeup of soil organisms. Each has its own combination of soil microflora, consisting of bacteria, fungi, nematodes, and insects, and each combination influences the population of pathogenic fungi in the soil, the amount of infections, and the expression of disease symptoms. Growers should learn the soil microflora "personality" of their nurseries.

NUTRITION

Nitrogen applications made too early promote damping-off. Germinating seed and new seedlings do not need much supplemental nutrition; the endosperm contains sufficient food to get seedlings well on their way.

MULCHES AND COVER CROPS

Cover crops grown and turned under just prior to sowing conifers may, depending on their type, retard or encourage damping-off problems. Legume cover crops promote large populations of damping-off fungi, grass crops somewhat smaller populations. Bare fallowing discourages the buildup of potential pathogenic fungi in the soil.

ASSAYS FOR SOILBORNE DISEASES

Assays for soilborne pathogens measure populations of particular fungi in the soil. Soil assays have been developed for *Pythium*, *Fusarium*, *Macrophomina*, and *Phytophthora* species (see the passage on monitoring of fungi in Chapter 33, Principles of Integrated Pest Management). Although population levels of these fungi indicate potential risk and the severity of disease in future crops, they are not reliable predictors of crop loss. The grower should use the assay as a warning signal to give an indication of potential problems and to help determine disease prevention measures, such as fumigation.

NURSERY DISEASE MANAGEMENT

Diseases in forest nurseries are best prevented because pathogens can spread very quickly and cause extensive damage in relatively short time periods within nurseries. Once disease symptoms appear, pathogen infection has usually been extensive and therapeutic treatments are often non-effective. Therefore, successful management of nursery diseases often involves reducing inoculum of potential pathogens in seedling production areas. Another major approach is to promote conditions which are non-conducive for pathogen infection are spread. Treating with chemical pesticides is often the last desirable and often last-implemented management approach, although sometimes this is the only way to prevent extensive losses.

Successful management of nursery diseases often involves reducing pathogen inoculum in seedling production areas.

I CULTURAL CONTROL

Continual monitoring of the seedling crop is important so that the first indications of disease can be determined and so that control efforts can be initiated promptly to reduce chance for pathogen spread.

Sanitation is an important aspect of managing diseases in forest nurseries. Many of the most important pathogens can reside saprophytically on many types of organic matter that may be present within seedling-growing areas. Removal of organic matter within greenhouse that may harbor pathogens is very important in reducing disease losses. Greenhouse interiors and reused styrofoam or plastic containers should be sterilized between crops to preclude carryover of pathogens onto new seedlings crops. Seedlings with disease symptoms should be periodically removed from both bare root and container stock to reduce chance for secondary spread of pathogens.

It is very important that pathogen-free seed be used to produce seedlings within nurseries. Some seedlots may require chemical treatments if

high levels of pathogens are present; all seedlots should be routinely treated with running-water rinses to help reduce pathogen surface contamination of seedcoats.

Controlling timing and amount of irrigation is very important in reducing losses from some diseases such as those caused by *Cylindrocarpon*, *Pythium*, and *Phytophthora* spp. It is especially important that container-grown seedlings are not over-irrigated; persistently high levels of water in containers promote anaerobic development of pathogens which often results in extensive root decay. Irrigation should be applied only in the morning to allow foliage to dry quickly during the day to help control *Botrytis* blight.

Air circulation within greenhouse is important so that seedling foliage can rapidly dry after irrigation to help reduce losses from *Botrytis* and *Fusarium* spp., both of which may attack above-ground tissues.

Another way to reduce disease losses is by restricting fertilizer during certain parts of the growth cycle. For example, nitrogen should not be applied to young, succulent seedlings when they are particularly susceptible to damping-off.

Diseases of bare root seedlings can be reduced by bare fallowing fields for one or more years between seedling crops. If cover or green manure crops are grown between seedling crops, fields must either be subsequently fallowed or fumigated prior to sowing new seedling crops because most pathogen populations increase on organic matter produced by cover crops. Rotating different seedling species among fields also helps reduce pathogen buildup within soils.

When seedlings are lifted from either production fields or containers, they must be carefully examined for indications of disease. All seedlings with disease symptoms should be culled during the packing process.

II BIOLOGICAL CONTROL

Some commercially-available biocontrol formulations – biofungicides developed for other agricultural crops show promise in forest nurseries. These are made up of either fungi or bacteria that are antagonistic towards pathogens. Biocontrol formulations are usually applied early in the growing cycle. They are either incorporated into soil-less growing media, which is made up of mixtures of peat moss with other organic or non-organic materials, or they can be applied directly adjacent to seed during sowing. Some biocontrol agents are applied directly to seed prior to sowing.

Some ectomycorrhizae are also antagonistic toward pathogens. Commercially-available mycorrhizal preparations are available and can be applied several times during the seedling growing cycle.

Biological control formulations provide an environmentally-friendly alternative to chemical pesticides for control of some nursery pathogens.

The use of biological control agents (living microorganisms used to control pests) is gaining recognition as an alternative disease control. There

is effective use of bacteria, actinomycetes, and fungi as agents for biological control of soil-borne plant disease.

Actinomycetes are bacteria with fungus-like growth characteristics. Several isolates of the actinomycete *Streptomyces* have proved effective as biological control agents against soil-borne plant pathogens. A commercial product, Mycostop.RTM. biofungicide, contains an isolate of *S. griseoviridis* as its active ingredient. That product is effective as a seed and soil treatment against seed rots, root and stem rots, and wilt diseases of various ornamental plants, caused by *Fusarium* spp. and other fungi. (Lahdenpera, et al. (1991). The Mycostop.RTM. Biofungicide Directions for Use (Kemira Biotech, Helsinki, Finland) recommends Mycostop.RTM. for use on pine and other conifers.

Another *Streptomyces* sp. isolate, is effective as a seed treatment against damping-off caused by *Pythium* spp. That patent also described some inhibitory activity against *Fusarium* spp. growing in agar-solidified growth media in petri plates.

Various fungi have been utilized as biological control agents to control fungal plant pathogens. Several isolates of *Trichoderma* spp. have also been employed to control soil-borne diseases, including *Fusarium* spp. on cotton.

Mycorrhizae are fungi which infect and form mutualistic relationships with plant roots. These fungi can improve plant growth by increasing the plant's assimilation of nutrients, especially phosphorus, which are sparingly soluble in the soil. Mycorrhizal infection will often make the plant roots more resistant to various soil-borne fungal pathogens. There are two major types of mycorrhizae: vesicular-arbuscular (VA) mycorrhizae, which infect most cultivated plants and produce specialized structures (vesicles or arbuscules) in the root cells, and ectomycorrhizae, which infect many forest trees such as pines and other conifers. Compositions and methods have been developed to help efforts to artificially inoculate plants with mycorrhizae (Castellano, 1994).

Ectomycorrhizal fungi are generally capable of infecting many species of plants. The ectomycorrhizal fungus which has been the most extensively investigated, *Pitholithus* sp., has been used to infect several species of the following woody plants: pine (*Pinus*), oak (*Quercus*), acacia (*Acacia*), and eucalyptus (*Eucalyptus*). Thus, ectomycorrhizal fungi can be generally considered to be somewhat nonspecific in the plants they infect.

Both VA mycorrhizae and ectomycorrhizae have been utilized as biological control agents, with limited success (Linderman,1994).

Ectomycorrhizae have shown some promise in controlling soil-borne diseases on conifer seedlings, but the protection to date has been unreliable due to the extreme variability of results. For example, *Laccaria* spp. exhibited limited control against *Fusarium* root rot and damping off on Douglas fir (Strobel and W.A.Sinclair ,1991), and pine (Chakravarty and S. F. Hwang, 1991), and *Paxillus involutus* increased resistance of pine

seedlings by 47% to *Fusarium* root diseases (Duchesne, et al. ,1988). Because of the limited and conditional control exhibited in these studies, the authors have expressed pessimism that they could be used effectively without further extensive research.

The present invention addresses a long felt need to provide an alternative to chemical control methods by utilizing a strategy employing novel ectomycorrhizae and *Streptomyces* isolates alone and in combination to effectively control conifer seedling diseases caused by *Fusarium*.

III CHEMICAL PESTICIDES

Chemical pesticides are usually applied as a last resort. In order to use the right chemicals, it is important to properly diagnose pathogens prior to treatments. Certain chemicals are only effective against certain groups of pathogens. For example, metalaxyl only controls oomycete pathogens (*Pythium*, *Phytophthora*) and is not effective against other pathogens such as *Fusarium*. Chemicals should be applied according to label instructions for timing and dosage rates.

It is best to rotate pesticides in order to reduce chances for pathogens to develop resistance. Selected pesticides should have different modes of action to limit chances for genetic mutations of pathogens.

Most pesticides are not effective therapeutically, they help prevent pathogen infection and colonization rather than kill pathogens that are already colonizing hosts. Pesticide applications just prior to lifting may be important to preclude fungal development during cold storage.

Chemical pesticides should only be used if other ways to control diseases are ineffective.

1. SOIL FUMIGATION

Fumigating soil prior to sowing is a common practice in nurseries. Several different materials have been used successfully, including dazomet, methylisothiocyanate/1,3-dichloropropene, and mixtures of methyl bromide and chloropicrin. Fumigation decreases *Fusarium* and *Pythium* populations sometimes to near zero. Methyl bromide with 33 percent chloropicrin will hold these pathogens in check for most of the first growing season. Follow-up disease control is done as needed with a carefully prescribed fungicide application plan.

Methyl bromide-chloropicrin soil fumigation may not be allowed after the year 2000 because its use is viewed as an environmental hazard (Smith and S. W. Fraedrich, 1993). Thus, alternatives are needed for controlling *Fusarium* diseases in trees.

2. FUNGICIDES

Treatment of seed with fungicides is not recommended. In previous years seed treatment was customary, but fungicides applied to the seed coat offer little or no protection to the emerging seedling. In addition, some seed treatments are phytotoxic. Although the effectiveness of fungicides to control damping-off is highly variable, many growers use them. Several fungicides are registered for use in forest nurseries to control soilborne diseases. Certain fungicides or combinations of fungicides seem to work better in one nursery than another. The fungicide metalaxyl has systemic properties and may be used prior to sowing to reduce populations of *Pythium* and *Phytophthora* in the soil. Metalaxyl is available in granular and liquid formulations. The first post-plant fungicide application should be made when most seedlings have emerged and the seeds begin to drop from cotyledon leaves. A good all-purpose preventive treatment for damping-off is a 50-50 mixture of captan and benomyl applied as a drench at rates recommended on the label. If frequent applications are planned, alternation of the captan-benomyl mix with other fungicides is advised to minimize the buildup of resistant pathogens.

With a 10-fold increase in seedling production occurring in the last few years, interest in the production and planting of longleaf pine (*Pinus palustris* Mill.) seedlings has reached an all time high. A limitation in producing even more seedlings is lack of high-quality seeds that not only germinate well, but result in plantable stock. Earlier results have shown that longleaf seed coats carry pathogenic fungi that not only reduce germination, but also result in significant seedling mortality (Barnett and others 1999). Pawuk (1978) and Fraedrich and Dwinell (1996) found that *Fusarium* sp. are commonly found on longleaf pine seeds and cause longleaf seedling mortality. Tests have shown that treating longleaf seeds with a sterilant or fungicide prior to sowing can improve both germination and seedling establishment (Barnett 1976, Barnett and Pesacreta 1993, Littke and others 1997). However, the effects of using both seed pretreatments to control seed-coat pathogens and fungicides to minimize seedling losses during the cultural period have not been reported. Our objectives were to develop recommendations for presowing treatments and fungicidal applications that will improve the efficiency of seedling production.

Results from this study demonstrate the effectiveness of reducing fungal populations on longleaf pine seed coats before they are sown in containers. Elimination of pathogenic fungi from seed coats increases seedling establishment and reduces sources of disease infestation later in the nursery cultural period. Although 30-percent hydrogen peroxide is labeled as a stimulant of pine seed germination, earlier research has shown that a 10-minute Benlate® seed drench was equally effective and is a safer means of reducing seed coat pathogens (Barnett and others 1999). Other fungicidal

chemicals or methodologies also may be effective if they are not phytotoxic to seed germination.

Combing presowing seed treatments to reduce pathogenic fungi on the seed coats with the application of appropriate fungicides to seedlings during the growing season to control pathogenic fungi greatly increases the efficiency of container seedling production.

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