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Efficacy of Some Fungicides in Parasite Suppression on Poplar Leaves (*Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora* spp.

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Abstract – The efficacy of fungicides in suppression of diseases caused by *Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora* spp. on poplar leaves is presented in this paper. The suppression was performed using three newer fungicides, which have not been studied in suppression of mentioned diseases in Serbia as yet (products Quadris, Equation Pro WG and Perfit). Also, the Copper lime 50 was used as test fungicide, whose efficacy was established previously by numerous authors. During biennial research in stoolbeds of clones *Pannonia* (*Populus x euramericana)* and S 6-36 (*Populus deltoides)*, satisfactory results were obtained for suppression of both fungus *Marssonina brunnea* and *Melampsora spp*. using Quadris and Copper lime 50, while fungicides Equation Pro WG and Perfit showed significantly lower activity. This conclusion was based on the average number of acervules, i.e. uredosoruses per cm² leaf surface, formed on treated plants. During both years, no significant differences were found between treated and untreated 1-year-old plants in diameter and height increment.

***Marssonina brunnea* / *Melampsora* spp. / poplar clones / chemical suppression / fungicides**

Kivonat – Fungicidek hatásossága nyárak levélkórokozóinak visszaszorításában (*Marssonina brunnea* (Ell. et Ev.) P. Magn. és *Melampsora* spp.). A dolgozat fungicidek hatásossági vizsgálatát tartalmazza a nyárak levelén kórokozó *Marssonina brunnea* és *Melampsora* fajok visszaszorításában. Három olyan új gombaölő szert használtunk, amelyeknek hatásosságát az említett betegségekre még nem vizsgálták Szerbiában (Quadris, Equation Pro WG és Perfit). Kontrollként a Copper lime 50 szert alkalmaztuk, amelynek hatásosságát előzőleg több szerző is megállapította. A kétéves kísérlet során Pannónia (*Populus x euramericana)* és S 6-36 (*Populus deltoides)* klónok anyatelepein a Quadris és a Copper lime 50 szerekkel mind a *Marssonina brunnea* mind a *Melampsora* fajok ellen kielégítő eredményeket értünk el, míg az Equation Pro WG és Perfit szerek szignifikánsan alacsonyabb aktivitást mutattak. A kiértékelést a kezelt növények cm²-nyi levélfelületén képződött acervuluszok, illetve uredo telepek átlagos száma alapján végeztük. A két év során nem találtunk szignifikáns különbséget a kezelt és kezeletlen egyéves növények átmérő- és magassági növedékében.

***Marssonina brunnea* / *Melampsora* spp. / nyár klónok / kémiai védekezés / fungicidek**

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1 INTRODUCTION

During the past decades, along with permanent attempts to define or create poplar genotypes less exposed to the leaf diseases attack (*Marssonina brunnea* and *Melampsora* spp.) and characterized by other desirable traits, possibilities of direct suppression of these fungi by chemical compounds were permanently studied. Considering the fact that poplar clones characterized by high susceptibility to these parasites are grown in our country, it is necessary to protect them using chemical products. According to numerous authors, a few fungicide treatments in nurseries and newly established plantations of poplars could provide satisfactory plant protection (Cellerino 1966, 1969, Castellani – Cellerino 1967, Gojković 1970, 1971, Avramović et al. 1991, 1997; Gojković-Avramović 1996, Keča 2003). Furthermore, chemical protection should be applied when favourable conditions for appearance and spreading of these fungi arise, when cultivating plants without optimal conditions, etc. In these cases, chemical protection is used in addition to basic (preventive) activities, related to selection of cultivars less susceptible to these diseases (Gojković – Avramović 1996). Applied in plantations, these chemical products could also suppress other diseases that occur on poplar plants. The absence of fungicide treatment during one year will not result in significant reduction of plant increment and plants decline, it will be accompanied with physiological weakening of plants, resulting in possible increase of other pathogens attack, such as *Dothichiza populea*. According to investigations of authors who studied the effects of these diseases upon plant development during two or more years (Castellani – Cellerino 1967; Avramović 1997, Keča 2003), only multiannual attacks of parasites on leaves lead to the reduction of plant height and diameter increment, resulting in decrease of total volume increment. Drying of individual trees in plantations occurs during intensive multiannual attacks.

The aim of the present study was to explore efficacy of some newer and one test fungicides in suppression of diseases on poplar leaves, caused by *Marssonina brunnea* (Ell. et Ev.) P. Magn. and *Melampsora spp.* There was an idea to protect leaves from these pathogens by several fungicide treatments, starting from the time of sprouting to the end of vegetative period. Also, differences in height and diameter increment between treated and untreated plants will be determined, due to plant height and diameter measurements.

2 MATERIALS AND METHODS

The possibility of parasites suppression on poplar leaves (*Marssonina brunnea* and *Melampsora* spp.) was tested in stoolbeds of poplars, established during year 2004 and 2005 at Experimental Estate of the Institute of Lowland Forestry and Environment (*Figure 1*). Two tested clones were present at experimental fields: clone *Pannonia* **(***P. x euramericana)* (*Figure 2*) and S 6-36 (*P. deltoides).*

The field trials were created in triplicate for each treatment and control (untreated plants), using complete random block design. Rows formed of twelve plants were used as replications. The spacing of planted cuttings in rows was 30 cm, while spacing between rows amounted 80 cm. Protective rows, formed of the I-214 clone, were established in order to prevent unwanted drift and deposition of fungicide on plants in individual treatments, as well as in control.

Figure 1. Experiment in 2005

Figure 2. Stoolbed of the Pannonia clone

The protection was performed using three newer fungicides, which have not been studied in restraint of mentioned diseases on poplars in Serbia as yet (products Quadris, Equation Pro WG and Perfit). Also, the Copper lime 50 was used as test fungicide, whose efficacy was established previously (*Table 1*). Application of active substances was done by backpack sprayer CP 3 (Cooper, Pegler and Co Ltd).

Table 1. Fungicides, their active substances and applied concentrations

Product	Active substance	Concentration of product
Copper lime 50	Copper oxichloride	0,5 %
Quadris	azoxistrobine	0,02 %
Equation Pro WG	famoxadone+cymoxanil	400 g/ha
Perfit	Hydrogen peroxide	0,4 %

Six fungicide treatments were performed in 2004: on June 1st and 22nd, July 8th and 28th, August 23rd and September 8th. Nine treatments were performed in 2005: on May 30th, June 14th and 24th, July 7th, 22nd and 30th, August 8th and 18th and September 7th. Number of treatments in studied years was determined according to reports and work of service for diagnostic and prognosis.

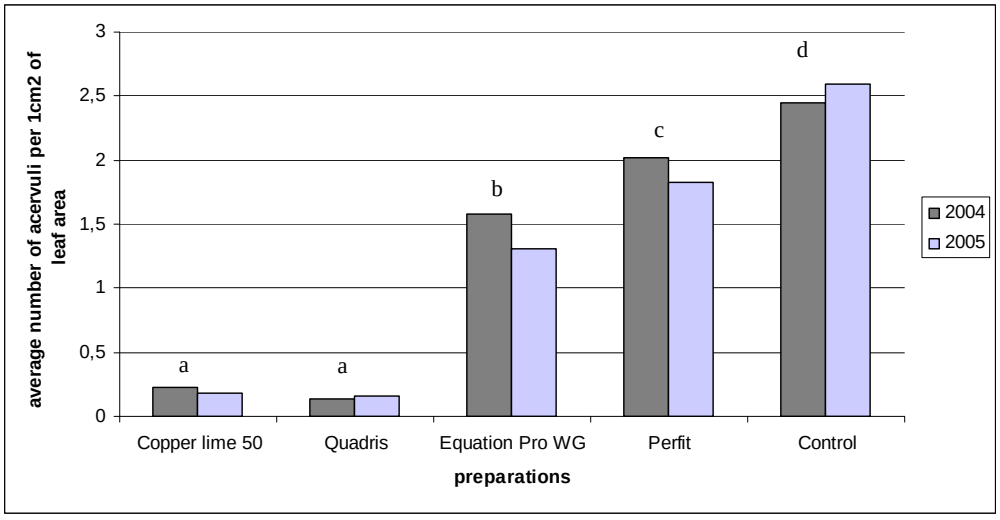
The final evaluation of applied fungicides efficacy in prevention of *Marssonina brunnea* and *Melampsora* spp. on plant leaves was done in the last decade of September, in both years. Five leaves from down, middle and above part of the crown were sampled from each seedling (15 in total). Acervules and uredosoruses were counted on each leaf, on five correctly arranged areas of 1 cm^2. During the data processing, sums were calculated for all five examined areas, and average number of fruiting bodies was expressed per cm^2 leaf area (Keča 2003). Also, the average number of fruiting bodies per cm^2 leaf area was calculated for each plant. Elements of plant growth (diameter and height) were measured during the overwinter period. Diameter of seedlings was measured at the plant base (accuracy 1 mm), while plant height with accuracy of 5 cm.

Results concerning efficacy of studied products were processed using the analysis of variance, and their relations are shown using Duncan´s test.

3 RESULTS

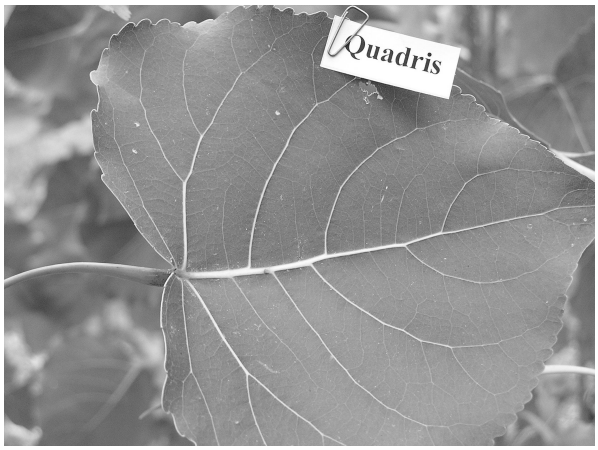
3.1 Fungicides efficacy in suppression of fungus *Marssonina brunnea*

On the leaves of *Pannonia* clone, all products showed significant activity in *M. brunnea* suppression, during both experimental years (*Figure 3*). Manifold higher activity to this pathogen showed Copper lime 50 and Quadris. Efficacy of these products was not significantly different, as well as the number of fruiting bodies between them, in both years. Lower level of efficacy was estimated for products Equation Pro WG and Perfit, mirrored in Duncan's test (*Figure 3 and 4*).



2004: F (for treatments)=140,6***, replications 0,10 ns;
2005: F (for treatments)=223,7***, replications 0,14 ns

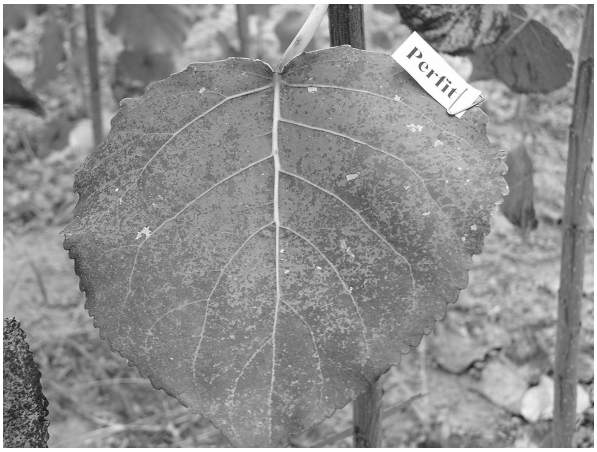
Figure 3. Effect of fungicides upon intensity of M. brunnea emergence on the Pannonia clone leaves



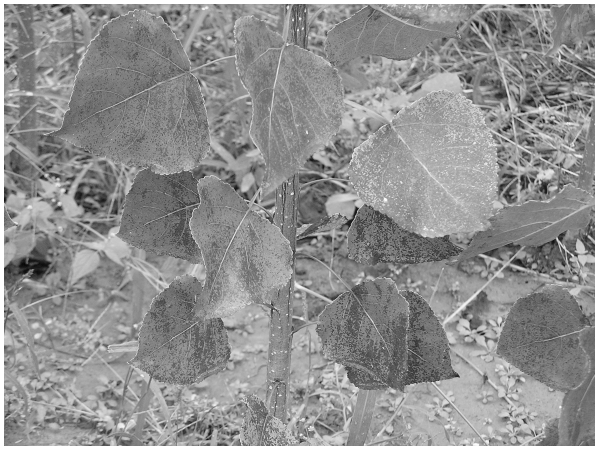
a) Quadris

b) Copper lime 50

c) Equation Pro WG



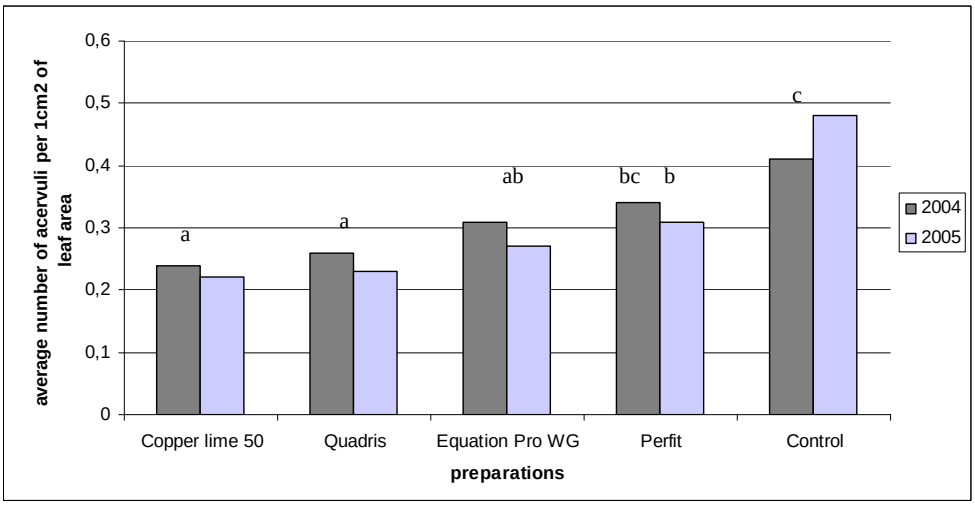
d) Perfit



e) Control

Figure 4a-e. Presence of M. brunnea acervules on the Pannonia clone leaves, in treated and control plants

Similar efficacy of fungicides in *M. brunnea* suppression was recorded on the leaves of S 6-36 clone. Therefore, the best protection of leaves was obtained with Copper lime 50 and Quadris. Lower efficacy was recorded for Equation Pro WG, while the lowest performance had Perfit, whose efficacy in 2004 was not significant, when compared with control plants (*Figure 5*).



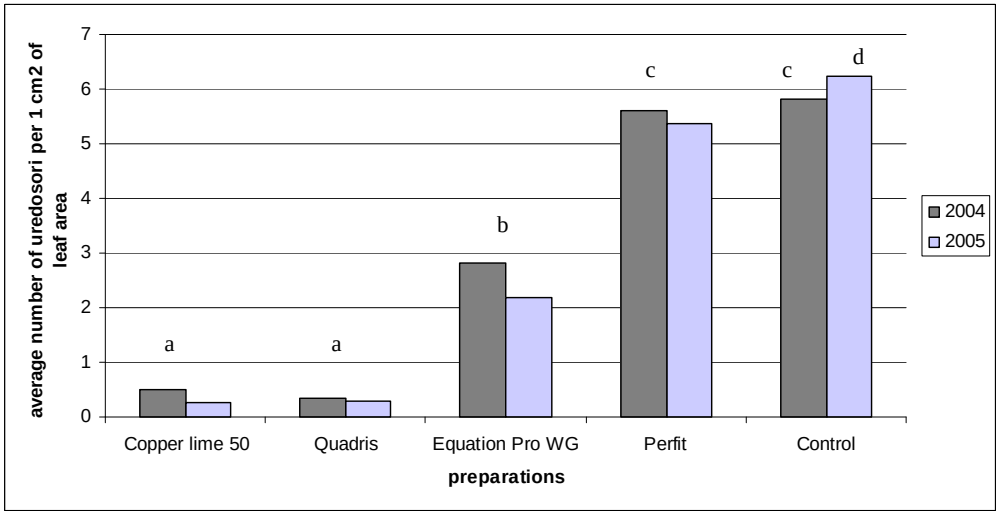
2004: F (for treatments) = 8,2***, replications 0,95 ns;
2005: F (for treatments) = 22,3***, replications 0,52 ns

Figure 5. Effect of fungicide treatments upon intensity of M. brunnea emergence on the S 6-36 clone leaves

3.2 Fungicides efficacy in suppression of fungus from the genus *Melampsora spp.*

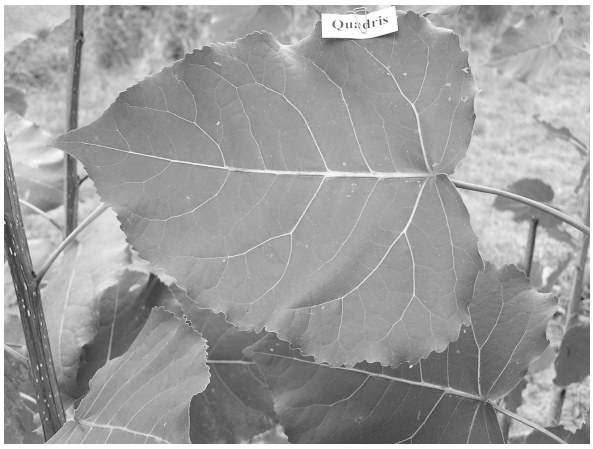
During two years, fungi from the genus *Melampsora* were not abundant on the *Pannonia* clone leaves. This is also confirmed by low average number of uredosoruses per 1cm² leaf area. During the final evaluation, uredosoruses were not found on seedlings treated with Copper lime 50 and Quadris in both years. At other treatments and the control uredosoruses occurred sporadically, so data were not shown in this report.

Products Copper lime 50 and Quadris exhibited very high efficacy in suppression fungi from the genus *Melampsora*, which is confirmed by low number of uredosoruses on the S 6-36 clone leaves in both years (*Figure 6 and 7*). Product Equation Pro WG had significantly lower efficacy, while the lowest activity showed Perfit. Inefficiency of Perfit was more pronounced in 2004, when significant differences were not found between treated and control plants, due to intensity of attack, i.e. number of fruiting bodies.



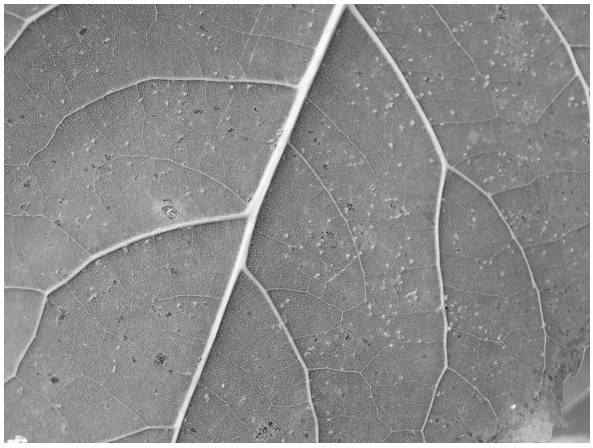
2004: F (for treatments) = 393,8***, replications 0,35 ns;
2005: F (for treatments) = 921,7***, replications 0,29 ns

Figure 6. Effect of fungicides upon intensity of Melampsora spp. emergence on the S 6-36 clone leaves



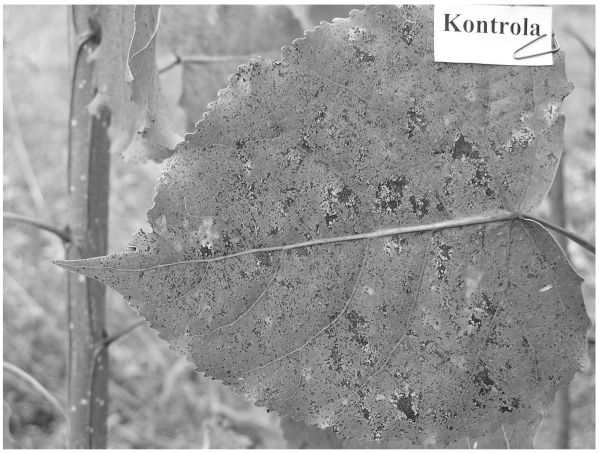
a) Quadris

b) Copper lime 50



c) Equation Pro WG

d) Perfit



e) Control

Figure 7. Presence of Melampsora spp. uredosoruses on the S 6-36 clone leaves in treated and control plants

Results presented in figures indicated the same efficacy of Copper lime 50 and Quadris in suppression of both leaf pathogens. Also, obtained differences between these two products were not significant. Also, it should be emphasized that in all cases considerable differences between replications were not found.

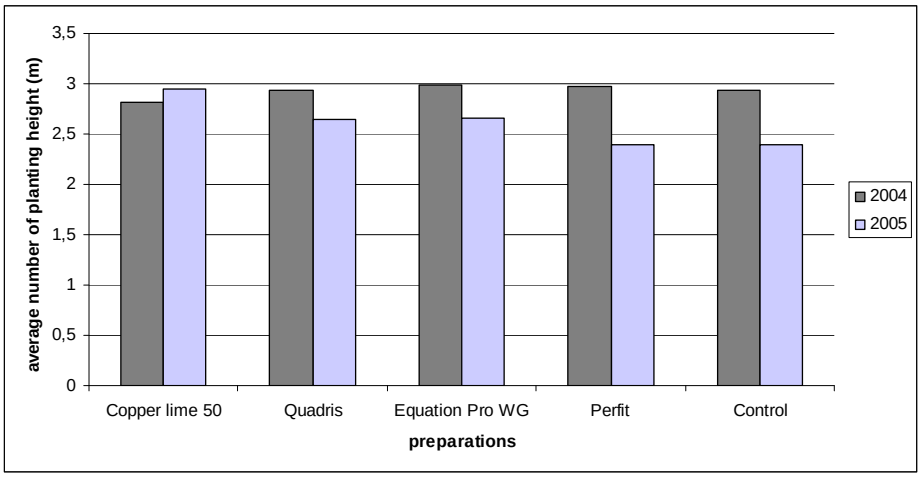
Furthermore, it was clearly shown that average numbers of fruiting bodies of both pathogens were lower on leaves of treated plants during 2005. Leaves protection was better in

2005 than in 2004, due to more abundant fungicide treatments in 2005, with shorter time intervals between them. Taking into consideration meteorological conditions, especially amount of precipitation, it may be concluded that these conditions were favorable for leaf diseases in both years. But, a greater amounts of precipitations during vegetative period in year 2005 (51 mm more than in 2004) probably facilitated appearance of a greater number of fruiting bodies on leaves of untreated plants (*Table 2*, *Figures 3, 5 and 6*).

Table 2. Amounts of precipitations (mm) in the period from April to September in year 2004 and 2005

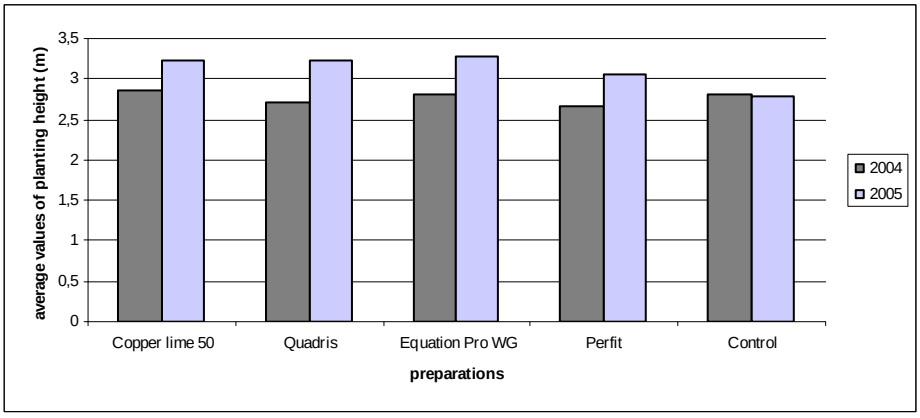
Year	Month						Total
	April	May	June	July	August	September	
2004	112	89	97	63	39	42	442
2005	65	67	68	94	138	61	493

Results of seedlings height measurements at the end of both experimental years indicated some differences between average values obtained for individual treatments. Analysis of variance showed no statistically significant differences, neither between individual fungicides, nor between control and treated plants (*Figures 8 and 9*).



2004: F (for treatments) = 1,33 ns, replications 0,24 ns;
2005: F (for treatments) = 0,59 ns, replications 0,08ns

Figure. 8 Average height of treated and untreated seedlings of Pannonia clone



2004: F (for treatments) = 0,41 ns, replications 0,61 ns;
2005: F (for treatments) = 1,74 ns, replications 0,57 ns

Figure 9. Average height of treated and untreated seedlings of S6-36 clone

Besides plant height, in order to accomplish the most complete impression about fungicide treatment upon plant development, plant diameter measurements were also used. According to analysis of variance, there were no significant differences between control and treated plants (data not shown).

4 DISCUSSION

According to numerous literature data, parasites on poplar leaves (*Marssonina brunnea* and *Melampsora* spp.) belong to the group of economic important pathogens. The long-term problems, caused by appearance of these diseases on poplar leaves, directed attention of scientists to explore possibilities of their suppression using chemical compounds.

During experiment planning, four fungicides of different chemical composition were selected. The Copper lime 50 (product from the group of copper compounds) is used because of its stability and efficacy in managing a wide range of fungal diseases. Quadris is a fungicide from the group of strobilurins, and it is effective against numerous diseases in both agriculture and silviculture. But, the resistance occurrence presents a serious problem in the use of strobilurines. Since 1998, several pathogens have developed resistance to strobilurins in the field (Kuck – Mehl 2003). Equation Pro WG is a fungicide that contains two active substances (famoxadone+cymoxanil), primarily labeled for managing pathogens that cause leaf rust on vegetable crops and grapevine. Hydrogen peroxide is formulated as a domestic product Perfit. It is used for soil, tools and equipment disinfection, in order to suppress pathogens responsible for flattening of sprouts.

Considering the importance of the problem, many authors have been interested in suppression poplar leaf diseases by fungicides.

During the sixties, numerous products were tested in various concentrations in poplars protection from the *Melampsora* species (Gojković – Vujić 1966). The best results in experiments concerning chemical suppression of leaf rusts, lasting for three years (1964-1966) in nursery production, were obtained with Copper lime 25, while somewhat lower efficacy had Maneb. Results with other fungicides were not satisfactory. A very high efficacy of the Copper oxichloride in treating fungi from the genus *Melampsora* on the leaves of sensitive clone S 6-36 was confirmed in our experiment.

Chemical suppression of *M. brunnea* has been examined in details during the sixties, in Italy (Cellerino 1969). Great complexes of poplar plantations were treated from both ground and air, in order to investigate efficacy and duration of protection, considering number and timing of treatments. The best results were obtained with products Ditan M-45 and Maneb. Furthermore, author reported that plantations of moderately sensitive clones needed two to three treatments per year in strictly defined time intervals, while sensitive clones required additional treatment.

In the late sixties, chemical protection of poplars from fungus *M. bunnea* has been studied by Gojković (1970). Considering previous results and his own three year long experiments in nursery plantations, he concluded that the most effective protection was achieved with Ditan 45, Maneb and Copper lime. Author especially emphasized importance of prevention, and in particular, early spring treatments. Experiments revealed acceptable results in plants protection in nurseries, using three or more applications. In comparison with other products used, Quadris and Copper lime showed considerably higher degree of efficacy in treating *M. brunnea* in our experiments.

Avramović et al. (1991) evaluated efficacy of nineteen fungicide compounds and their combinations in treating fungus *M. brunnea*. Experiments were conducted in nurseries situated at different localities, during the period from 1983 to 1986. According to their results,

bitertanol provided the best protection of leaves, while satisfactory effects were obtained for fungicides containing triphorin, copper oxichloride, benomyl and carbedazim, as well as following combinations: triphorin+propineb, benomyl+propineb and tiophanate+propineb.

According to two year long researches, various efficacies of fungicides in managing *M. brunnea* and *Melampsora* spp. were reported by Keča (2003). During the year 2000, which was centenary extreme considering low rainfall and high daily mean temperatures, the best results were recorded for Benomyl WP-50. The most effective product in the rainy year 2001 was Copper lime, due to its longevity and steadiness on leaves. Along with high efficacy of Copper oxichloride, its steadiness was also evident in our study, considering that climate events during 2004 and 2005 were similar to those in 2001.

Statistically significant differences were not found in height and diameter increment between treated and control plants, although leaves of untreated plants were exposed to intensive attack of studied fungi. Similar results were reported by Avramović (1997) and Keča (2003), when crowns of poplar plants in newly established plantations were repeatedly treated with different fungicides during two years. These authors approved that juvenile plants, exposed to moderate attack of these fungi, do not exhibit significant reduction of height and diameter increment. It may be concluded that these leaf diseases show cumulative effects, while consequences become evident in the following years, when trees are older (Castellani – Cellerino 1967, Cellerino 1969). In our experiment, considerable variations of growth elements were not found, probably due to a strong influence of other factors (the juvenile phase of plants, favourable climate conditions, clones tolerance to these diseases, etc.). These factors affected plants stronger than parasites.

Taking into consideration our Institute's results related to clones susceptibility to *M. brunnea* and *Melampsora spp.*, collected during several years (Avramović et al. 1998, Pap et al. 2006), clones used in our experiments exhibited different sensitivity to the major leaf diseases of poplars. According to these authors, clone *Pannonia* belongs to the group of moderately sensitive clones to *M. brunnea* and insignificantly sensitive to fungi from the genus *Melampsora spp.* Clone S 6-36 is labeled as very sensitive to the rust and a little sensitive to fungus *M. brunnea*. Sensitivity of clones obtained in our study is in accordance with results of authors mentioned above.

Number of treatments, necessary for safe plant protection in nurseries, was not investigated. According to obtained results, it could be concluded that at least 6-8 fungicide treatments, applied in appropriate time intervals, are necessary for sensitive clones under favorable climate conditions for development and spreading of these diseases. Complete plant protection could probably be achieved even with fewer treatments, but the main periods of spores dispersion must be precisely determined for both pathogens. Plants protection must be simultaneous with the major period of leaves infection, in order to accomplish satisfactory protection of nursery with low number of treatments.

5 CONCLUSIONS

Following conclusions could be drawn from biennial research:

In stoolbeds of poplar clones *Pannonia* and S6-36, satisfactory suppression of both *M. brunnea* and fungi from the genus *Melampsora spp*. were performed with Quadris and Copper lime 50, while lower efficacy was obtained with fungicides Equation Pro WG and Perfit.

Products Quadris and Copper lime 50 are recommended for treating *M. brunnea* and *Melampsora* spp. in nurseries and newly established plantations, while poorly effective fungicides Equation Pro WG and Perfit should be avoided.

Successive protection of plants from studied pathogens in nurseries could be achieved by 6-8 fungicide treatments during the vegetative period.

Statistically significant differences were not found between treated and control one-year-old plants in relation to height and diameter increment in both experimental years.

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