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ANALYSIS OF SCOTS PINE (*PINUS SYLVESTRIS* L.) SEEDLING SURVIVAL DEPENDING ON SOWING TIME AFTER MICROWAVE STERILISATION OF SUBSTRATE

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Abstract. Soil sterilisation has a great influence on planting stock quality and quantity due to significant accumulation of infectious material, especially in permanent forest nurseries. In the experiment microwave oven was used as a tool for disinfection of samples in small pots (volume 330 cm3 each). Peat substrate and mineral soil were used during disinfection. Scots pine (*Pinus sylvestris* L.) was chosen for seedling survival analyses as this tree species is the most common one in Polish forests and its seedlings are susceptible to diseases caused by damping-off fungi. In the experiment a negative influence of microwave radiation on seedling survival was observed. Seedlings in the pots sown shortly after disinfection died or had poor vitality. In the case of the peat substrate, faster germination and lower mortality was observed in pots sown after 30 days since the treatment. Soil pots in general were characterised by higher mortality of seedlings than the pots with peat. High concentration of infectious material in soil samples quickly killed all the seedlings in the control group. Microwave radiation had a negative impact on seedlings growing on the soil substrate, if the seed were sown too early. In the pots sown on the day of disinfection only 60% of seeds germinated and only few seedlings survived until the end of experiment. Results show that the grace period is longer for the substrate based on peat and lasts 30 days, whereas for mineral soil it lasts 23 days. Sowing at the appropriate time led to a significant increase in the number of seedlings.

Keywords: disinfection, microwaves, forest nursery, soil sterilisation

INTRODUCTION

Every year infectious diseases cause enormous losses in forests. The most important of those include: infectious seedling rot, pine brown spot needle diseases, powdery mildew on oak trees, as well as those causing diseases of the root systems – root polypore and Armillaria root rot. Some of the diseases are hard to detect by the casual observer, yet they can interfere with the realisation of economic targets to a significant degree. Other diseases have a violent course, such as the infectious seedling rot which, additionally, is considered to be the most dangerous and inflicting the greatest damage in forest nursery production (Mańka 2005, 2011). Obviously, in the face of such a threat a number of actions are undertaken, of both prophylactic (choice of suitable sites for nursery, fertilisation) and intervention character (chemical agents and other measures). It is accepted that substrate disinfection is of key importance for the limitation of the rate of infestation with seedling rot. At present, the most popular measure applied in forestry in this respect is chemical protection. Every year a list of agents approved for application in forests is published. The number of registered agents and preparations systematically decreases, which makes chemical protection more difficult and less effective (Głowacka 2010). Additionally, the recent pro-ecological trends enforce a reduction of the application of such preparations. The ongoing process of certification in the National Forests has also led to a reduction in the number of chemical preparations approved for application, due to their high toxicity (Głaz *et al.* 2008). The biological methods available now are insufficiently effective, and they are applied mainly as a prophylactic measure. In addition, in the case of a high concentration of pathogens their effectiveness is too low. The method based on disinfection with steam is being applied, but it requires highly efficient sources of steam and a lot of time, which makes it economically non-viable (Rutkowski 1994). However, there is ongoing research on more efficient methods of application of steam for substrate disinfection. In view of those circumstances, it is no wonder that there is a search for new methods of disinfection, characterised by low costs and free on any negative effect on the natural environment.

Currently microwave radiation is used on a large scale for the sterilisation of e.g. food, medical equipment, medical wastes, and even accessories for infants (Ikediala *et al.*1999, Jakubowski 2008, Shiah *et al*. 2001). There is no doubt that microwave radiation kills viruses, fungi and bacteria (Hamid *et al*. 1968, Hurlock *et al.* 1979). Work is being continued on developing a microwave device for the sterilisation of soil substrate (Słowiński 2009a,b,c). Research conducted at the Department of Mechanisation of Forest Works, University of Agriculture in Kraków, demonstrated the effectiveness of microwave radiation in soil sterilisation and, consequently, in improving the growth and quality of seedlings (Słowiński 2010, Słowiński and Stępniewska 2010).

The small number of studies and publications devoted to the subject makes it difficult to make a credible estimation of the potential applicability of microwave sterilisation in forestry. It is worth noting, however, that microwaves may provide an alternative to the treatments applied currently.

In view of the above it was decided to test, under laboratory conditions, for two nursery substrates – peat and mineral soil, whether apart from the positive effect on germination of seedlings, which prior disinfection of the substrate appears to undoubtedly have, there will be no detrimental effects of microwaves on the substrate in the form of reduced survival rate of seedlings, whether the time of sowing relative to the time of disinfection will have an effect on the rate of survival, and whether there is a need to allow a waiting period between the time of microwave sterilisation of the substrate and the time of sowing.

MATERIAL AND METHOD

In order to ensure favourable and uniform conditions for the germination of seeds and for the growth of seedlings, the experiment was conducted in a phytotron that built specially for this study, using metal frame as the main structural element. To prevent excessive drying and to permit the creation of a suitable microclimate, the phytotron was screened on all sides with black plastic sheet. The entire process of seed germination and seedling growth was controlled. The conditions in the phytotron were as follows: temperature – 20^{o}C, light intensity – from 730 to 840 Lux, relative humidity – from 75 to 90%. To ensure a suitable level of illumination, the light phase was set at 12 hours (from 8:00 to 20:00) and the dark phase also at 12 hours (20:00 do 8:00). The required switching on and off of the lighting was controlled by a programmed time switch. The fluorescent tubes installed in the phytotron were designed for green plant cultures. The emitted wavelength corresponded to that absorbed by chlorophyll of plants. Pots with substrate after microwave sterilisation were placed in the phytotron prepared as above. The process of irrigation was fully automated. The irrigation system consisted of a 50 litre water tank, and a 12V water pump, connected with the sprinkler by means of tube 4 mm in diameter. The pump was switched on by means of a timer that initiated the process of irrigation at 10:00 in the morning and at 19:00; in each case the duration of the irrigation cycle was 60 seconds.

The experiment was conducted on two kinds of substrates commonly used in forest nurseries, with vastly differing properties: peat substrate and a mineral soil – weakly sandy loam. The peat-pearlite substrate, de-acidified with dolomite to pH 5.5, was produced in the Forest Division Rudy Raciborskie. The mineral soil was taken from the area of the Forest Division Niepołomice, in the forest nursery in Kłaj, from sites characterised by a high level of infestation of seedlings with root rot. The substrates were put into pots with volume of 330 cm^3 – the adopted substrate weights were 200 g for the mineral soil and 35 g for peat. For each experimental treatment 10 pots were used, 5 with peat and 5 with mineral soil. In addition, 10 pots were prepared with soil used to cover the seeds sown. In total, 130 pots were filled with the substrates (Photo 1). Substrate disinfection was conducted using a microwave oven, Electrolux model EMS 2840 S, microwave power of 900 W, frequency of 2.45 GHz, capacity 28 litres. All the pots that were to be disinfected were placed in the microwave over one by one. The time of irradiation of each pot was 2 minutes (120 s). After the over switched off, the pot was removed and after ca. 30 seconds temperature was measured at three different points on substrate surface using a pyrometer model VA 6510.

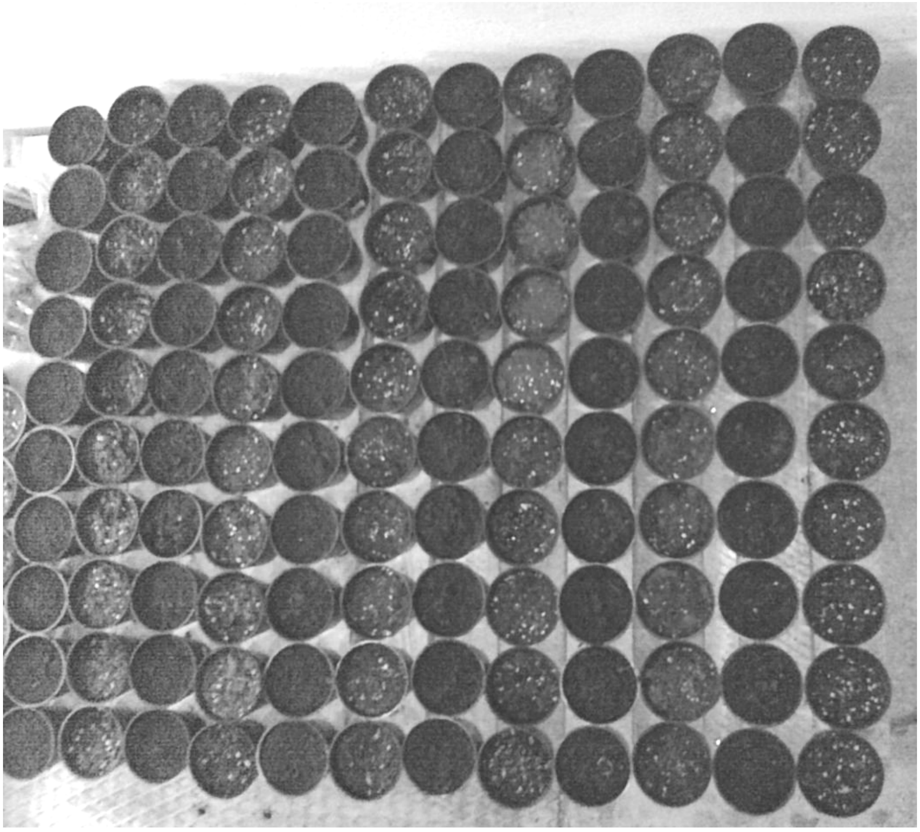

Photo 1. View of the pots filled with the substrates in the experiment

For the study on the rate of survival of seedlings seeds of the most popular species in Polish forests were chosen, i.e. Scots Pine (*Pinus sylvestris* L.). Moreover, the species is recommended for early detection of seedling rot pathogens, as a species susceptible to infections (Stocka 1997). The seeds were sown in substrates subjected to microwave radiation, at the following times: after cooling down of the substrate, and after 1, 2, 3, 5, 7, 10, 14, 18, 23 and 30 days. The control sample (without microwave disinfection) was sown with seeds on the first day of the experiment. Seeds of Scots Pine (*Pinus sylvestris* L.) were sown by hand, in the amount of 30 seeds in each pot. To prevent the seeds from being washed out during the irrigation, they were covered with a layer of substrate prepared in prior. After the sowing and covering of the seeds, each pot was marked with a label. The information replic on the labels replic the date of sowing and the identification number of the pot. This helped eliminate the possibility of making a mistake and facilitated exact observations. Records of the observations were kept for each pot separately. Based on the pot identification numbers, a count was made of the numbers of germs and seedlings, and then the data were recorded in the form of a table. The temperatures attained by the substrates in the pots and the results concerning the numbers of seedlings in the particular pots were subjected to statistical analysis. The statistical analyses were made using the StatSoft, Inc. STATISTICA ver. 9.0 data analysis software system, and the Microsoft Excel spreadsheet. The Mann-Whitney U-test was applied, the adopted level of statistical significance of differences being $p<0.05$.

RESULTS AND DISCUSSION

Substrate moisture, determined by means of a moisture analyser, was 54.4% for the peat substrate and 48.3% for the mineral soil.

Temperature measurement after microwave disinfection, made by means of a pyrometer, revealed that the mean temperature for the mineral soil was 67.7°C (standard deviation 3.29) and it was only slightly higher that attained by the peat substrate after the microwave treatment – 64.4°C (standard deviation 4.38). The temperature differences were statistically significant ($\alpha = 0.001$). In percentages it means that the mean temperature of the mineral soil was 5% higher than that of the peat substrate. As the temperature measurement was made after about 30 seconds from the microwave treatment, it should be assumed that the maximum temperature was higher. This means that during the disinfection, thanks to the high temperature, most of the phytopathogenic bacteria, *Fusarium* spp., grey rot (*Botrytis* spp.), *Rhizoctonia solani*, nematodes, *Pythium* spp., and *Phytophtora* spp. Should have been destroyed.

During the experiment a negative effect of microwave radiation was observed in the peat substrate. Samples sown too soon after the microwave disinfection were characterised by poorer vitality and died. The negative effect of microwave radiation was evidenced by the fact that the survival rate of seedlings sown on the day of the disinfection was distinctly lower than in the control sample, with statistically significant differences with relation to nearly all remaining experimental treatments (Tab. 1). The good results obtained for the control sample were probably due to small amounts of infectious material in that kind of substrate. According to the literature, peat substrate provides unfavourable conditions for the growth of fungi, which was reflected in the results of this experiment. However, one should not treat the peat substrate as one that is absolutely safe. The level of infestation is an effect of the level of infectious material which is very high in intensively used nurseries. Also other kinds of substrates, containing lower amounts of high peat, can be a cause of very high levels of infections.

Table. 1. Statistical significance of differences in the number of seedlings among the treatments: control – no microwave treatment, 0 day – sowing on the day of application of microwaves, 1 day – sowing after one day after application of microwaves to maximum number of grown seedlings germinated at different time intervals after microwave disinfection for peat substrates, x – statistically significantly fewer seedlings, **x** – statistically more seedlings, – no statistically significant differences

Time of sowing	Significance of results for the peat substrate, $\alpha = 0.05$		
	control – maximum	0 day – maximum	1 day – maximum
Control		x	–
0	**x**		**x**
1	**x**	x	
2	**x**	x	–
3	**x**	x	–
5	**x**	–	**x**
7	**x**	**x**	**x**
10	–	x	–
14	–	x	–
18	–	x	–
23	–	x	–
30	–	x	**x**

In spite of the conditions, unfavourable for the growth of pathogens, a positive effect of microwave radiation is clearly visible. The sample sown 30 days after the treatment was characterised by faster germination and a larger number of seedlings (Fig. 1). After about 4 weeks from the time of sowing, the rate of emergence in the sample sown a month after the disinfection stabilised at a constant high level till the end of the experiment. For comparison, during the same period the control sample had a number of seedlings lower by nearly 25%, while the highest number of seedlings in that sample was lower by over 6%. These results confirm that microwave radiation reduces the occurrence of seedling rot. The application of disinfection makes sense only when it results in the production of greater amount of seedling material of acceptable quality. This can be achieved by sowing seeds into a substrate when it no longer creates conditions noxious for seed germination. For the peat substrate, for achieving the best effect the sowing should be made not earlier than 18-30 days after microwave disinfection.

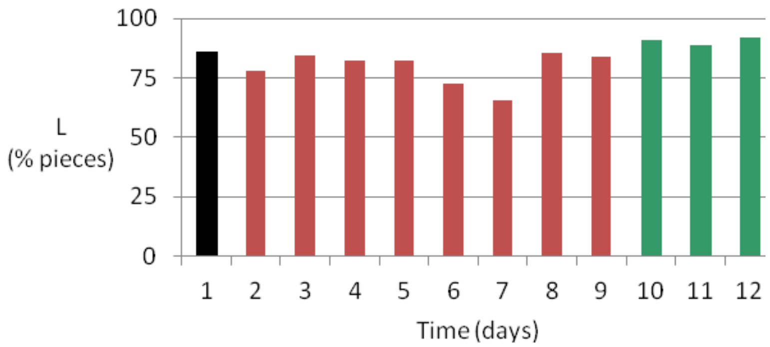

Fig. 1. Maximum percent of seedlings grown in peat substrates, L, depending on the time of sowing after microwave disinfection

The mineral soil was characterised by different properties than the peat substrate. As demonstrated by the results, the primary difference is in the degree of infestation by seedling rot which was considerably higher than in the case of the peat-based substrate. A negative effect of microwave radiation was also observed. In the sample sown on the day of the disinfection only 60% of the seeds germinated, and from those only a few seedlings survived till the end of the experiment! This proves how important is the correct time of sowing for achieving good emergence. High concentration of infectious material led to a rapid infestation and decay of seedlings in the control sample. At the end of the experiment the average number in the sample was only 10 seedlings. A positive effect of microwave disinfection was observable already in the case of sample sown on the second day after the treatment, where the average number of seedlings at the end of the experiment was nearly 20. Obviously, this does not mean that we should sow seeds after just one day, but rather that the disinfection played its intended role by killing rot pathogens. The experiment demonstrated that statistically significantly more seedlings emerged as a result of sowing after 14 days from microwave treatment with relation to: the control, sowing on the day of microwave disinfection, and sowing one day after the disinfection (Tab. 2). Sowing after 23 days from the microwave disinfection was characterised by very high numbers and quality of seedlings. The phenomenon of seedling dying did not appear, and the quality of the seedlings was stable at a high level (Fig. 2).

Table 2. Statistical significance of differences in the number of seedlings among the treatments: control – no microwave treatment, 0 day – sowing on the day of application of microwaves, 1 day – sowing after one day of application of microwaves to maximum number of grown seedlings germinated at different time intervals after microwave disinfection for mineral substrates, x – statistically significantly fewer seedlings, **x** – statistically more seedlings, – no statistically significant differences

Time of sowing	Significance of results for the mineral substrate, α = 0.05		
	control – maximum	0 day – maximum	1 day – maximum
Control		–	–
0	–		–
1	–	–	
2	–	–	–
3	x	**x**	**x**
5	–	–	–
7	–	–	–
10	–	**x**	–
14	**x**	**x**	**x**
18	**x**	**x**	**x**
23	**x**	**x**	**x**
30	**x**	**x**	**x**

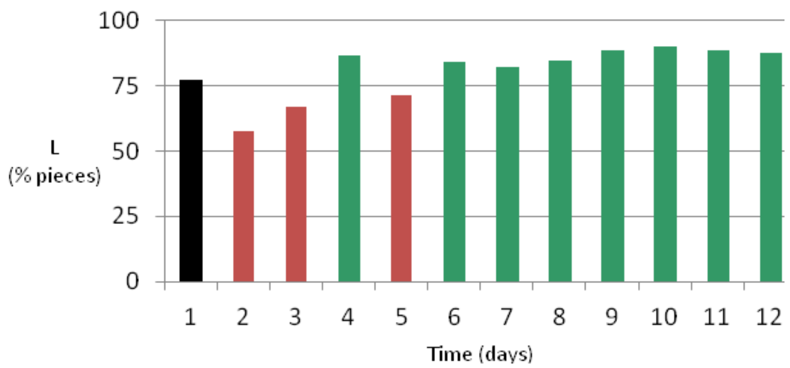

Fig. 2. Maximum percent of seedlings grown in the sandy loam soil, L, depending on the time of sowing after microwave disinfection

After 28 days the number of seedlings was over 10% higher than in the control sample, while the maximum number of seedlings increased by nearly 15%. Sowing on the day of disinfection caused the death of almost all seedlings over a longer period of time. To achieve the best effects of sowing on sandy loam, one should wait 23 days from the moment of microwave disinfection.

CONCLUSIONS

Based on the results obtained from the experiment the following conclusions were formulated:

1. Time of sowing after microwave disinfection has an effect on the survival rate of seedlings. Microwave disinfection may lead to a deterioration of emergence and to the death of seedlings if the sowing is made too soon after the treatment.
2. Seedlings growing on the peat substrate were infected by rot fungi to a lower extent compared with the sandy loam soil.
3. Sowing on the day of disinfection (after the substrate has cooled down) significantly reduces the survival rate of seedlings and should not be applied.
4. The experiment demonstrated that, in the case of the peat substrate, to achieve the best effects one should wait about a month before sowing seeds after microwave disinfection of the substrate. For the mineral soil, that time is shorter and amounts to 23 days.

REFERENCES

Głaz J., Zajączkowski G., Jabłoński M., 2008. Information on PGL Lasy Państwowe 2007 (in Polish). Druk-intro s.a.. ISSN 1641-3210.

Głowacka B., 2010. Agents for plant protection and decomposition of trunks of forest trees recommended for application in forestry in the year 2011 (in Polish). Copyright by Instytut Badawczy Leśnictwa, Sękocin Stary. Analizy I raporty. 15. ISBN 978-83-87647-96-4.

Hamid, M.A.K., Kashyap C.S., Cauwenberghe R.V., 1968. Control of grain insects by microwave power. Journal of Microwave Power, 3(3), 126-135.

Hurlock E.T., Llewelling B.E., Stables L.M., 1979. Microwaves can kill insect pests. Food Manufacture, 54(1), 37-39.

Ikediala, J.N., Tang J., Neven L.G., Drake S.R., 1999. Quarantine treatment of cherries using 915 MHz microwaves: Temperature mapping, codling moth mortality, and fruit quality. Postharvest Biology and Technology, 16(2), 127-137.

Jakubowski T., 2008. Effect of microwave field on the dynamics of changes in mass and temperature of potato tuber (in Polish). Inżynieria rolnicza, 6(104), 63-69.

Mańka K., 2005. Forest phytopathology (in Polish). PWRiL, ISBN 83-09-01793-6.

Mańka M., 2011. Diseases of forest trees (in Polish). PWRiL, ISBN 978-83-09-01071-5.

Rutkowski K., 1994. Energy consumption in thermal disinfection of greenhouse substrate (in Polish). Zesz. Probl. Post. Nauk Roln., 415, 321-328.

Shiah TC., Chang TT., Fu CH., 2001. The application of microwave irradiation for the disinfection of paper. Taiwan J. Forest Sci., 16, 327-332.

Słowiński K., 2009a. Use of microwave heating of forest nursery soil. Formec 2009. 42 International symposium on forestry mechanization. CULS Prague, 360-367. ISBN 978-80-213-1939-4.

Słowiński K., 2009b. Microwave device for soil heating. Mobilné energetické prostriedky – Hydraulika – ivotné prostredie – Ergonómia mobilnych strojov. Technická Univerzita vo Zvolene. 167-174. ISBN 978-80-228-2012-7.

Słowiński K., 2009c. Microwave device for soil disinfection (in Polish). Zesz. Probl. Post. Nauk Roln., 543, 319-325.

Słowiński K., 2010. Substrate disinfection with microwave radiation for the production of seedlings (in Polish). Prace Komisji Nauk Rolniczych, Leśnych I Weterynaryjnych PAU, 14, 133-141.

Słowiński K., Stępniewska H., 2010. Effect of microwave radiation on the level of pine seedlings infestation by Rhizoctonia solani and on the growth traits of the seedlings (in Polish). Prace Komisji Nauk Rolniczych, Leśnych I Weterynaryjnych PAU, 14, 143-152.

Stocka T., 1997. Seedling rot (in Polish). Biblioteczka Leśniczego, z. 88, Wydawnictwo Świat, Warszawa.

PRZEŻYWALNOŚĆ SIEWEK SOSNY ZWYCZAJNEJ *PINUS SYLVESTRIS* L. W ZALEŻNOŚCI OD CZASU SIEWU PO DEZYNFEKCJI MIKROFALOWEJ PODŁOŻA

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S t r e s z c z e n i e. Dezynfekcja podłoża ma bardzo duży wpływ na ilość i jakość materiału sadzeniowego. Powodem takiego stanu rzeczy jest znaczne nagromadzenie materiału infekcyjnego. Doświadczenie przeprowadzono z wykorzystaniem kuchenki mikrofalowej jako źródła mikrofal. Glebę napromieniowywano w doniczkach o pojemności 330 cm³. Badania wykonano dla dwóch rodzajów podłoży: substratu torfowego oraz gleby mineralnej. Do badań przeżywalności siewek wybrano nasiona najpopularniejszego gatunku w polskich lasach, jakim jest Sosna zwyczajna *Pinus sylvestris*. Ponadto gatunek ten, jako podatny na infekcje, jest zalecany do szybkiego wykrywania patogenów zgorzeli siewek,. W trakcie doświadczenia zaobserwowano negatywny wpływ promieniowania mikrofalowego. Siewki w doniczkach obsianych zbyt szybko po dezynfekcji charakteryzowały się gorszą żywotnością i zamierały. Dla substratu torfowego doniczki obsiane 30 dni po zabiegu charakteryzowały się szybszym kiełkowaniem oraz większą liczbą siewek. Dla gleby mineralnej główną różnicą w stosunku do substratu torfowego jest stopień porażenia przez zgorzel siewek, który jest znacznie wyższy niż w przypadku podłoża na bazie torfu. Zaobserwowano również szkodliwy wpływ promieniowania mikrofalowego. W doniczkach obsianych w dniu dezynfekcji wzeszło jedynie 60% nasion, z czego do końca doświadczenia przeżyło tylko kilka siewek. Duża koncentracja materiału infekcyjnego w tym podłożu doprowadziła do szybkiego porażenia i zamarcia siewek próby kontrolnej. Pozytywny wpływ dezynfekcji był widoczny w przypadku doniczek obsianych na drugi dzień po zabiegu. Z przeprowadzonych badań wynika, że dla substratu torfowego, w celu uzyskania lepszego efektu, powinno się odczekać około miesiąca przed wysiewem nasion, w przypadku wykonania zabiegu dezynfekcji mikrofalami. Dla gleby mineralnej czas ten jest krótszy i wynosi 23 dni.

S ł o w a k l u c z o w e: dezynfekcja, mikrofale, szkółka leśna, sterylizacja gleby