Int. Agrophysics, 2005, 19, 85-89

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Germination capacity and health status of alfalfa seeds after laser treatment**

M. Wilczek[1]*, *R. Koper*[2], *M. Ćwintal*[1], *and T. Korniłłowicz-Kowalska*[3]

[1]Department of Crop Production, [2]Department of Physics, [3]Department of Agricultural Microbiology, University of Agriculture, Akademicka 15, 20-934 Lublin, Poland

Received February 3, 2004; accepted August 16, 2004

A b s t r a c t. Laboratory studies on the germination of alfalfa seed (American variety 'Legend') were carried out completely randomly in four replications in 2002. The factors studied were as follows: 1. irradiation with divergent He-Ne laser light with a surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2} applied 1, 3 and 5 – times. 2. seed dressings: Funaben T, Sarfun T 65 DS and Super-Homai 70 DS in a controlled environment. The number of seeds germinating normally and abnormally as well as hard seeds and seeds infected with fungal disease was determined.

Seed treatment with He-Ne laser light did not significantly influence the share of those seeds germinating normally. However, it did decrease the percentage of those seeds germinating abnormally, the hard seeds and also those seeds infected with disease but only when double the power was used. Laser light with surface power density and multiplication of R6x3 and R6x5 destroyed fungi from the *Penicillium* kind completely, whereas in a dose of R3x3, R3x5 and R6x1 it significantly stimulated the growth of fungi from the *Alternaria* type. All seed dressings destroyed fungi from the *Penicillium* and also *Alternaria* types completely (except for Super-Homai 70 DS dressing).

K e y w o r d s: laser treatment, alfalfa, germination

INTRODUCTION

The most important features in seeds are their ability to germinate and their health status. Studies conducted so far on seed treatment with laser light have shown that this treatment increases the seed ability to germinate (Podleśny, 1997; 2002). This research was carried out in order to establish whether laser irradiation in respect of alfalfa seed of American origin is feasible. Its aim was to determine the influence of the laser treatment of seed on its ability to germinate, the share of hard seeds, and its influence on those seeds infected by disease when compared with the control and dressed seed. Moreover, the fungal strains infecting the seed cover were determined. The dearth of results quoted in the literature on this subject prompted the present authors to undertake this experiment.

METHODS

In 2002, laboratory studies with alfalfa seeds (*Medicago sativa* L ssp. *sativa*) var. 'Legend', multi-leaf form, were carried out. The following factors were examined: 1) irradiation with a divergent He-Ne laser beam with surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2}; 2) seed dressings: Funaben T, Sarfun T 65 DS and Super-Homai 70 DS in a controlled environment. The seeds were subjected to 1, 3 and 5 irradiation rounds on a laser device designed according to the Koper and Dygała patent (1994). A single irradiation round lasted for 0.1 s.

Germination of the seed was carried out according to the ISTA instructions (ISTA, 1999) on Petri dishes. Tissue paper soaked with water served as the substrate. One hundred seeds were tested on one dish. Germination took place at a temperature of about 20°C, and the number of seeds which had germinated was counted after 4 and 10 days. The number of normally and abnormally germinated seeds as well as the number of hard seeds and seeds infected with disease was determined. The experiment was carried out completely randomly in four replications. In order to identify the fungal strains inhabiting the alfalfa, seeds with

*Corresponding author's e-mail: fizar19@ursus.ar.lublin.pl
**This work was supported by the State Committee for Scientific Research, Poland, Grant No. 6 PO6R 03521.

mould were transferred onto a dish with Martin's substrate (Martin, 1967) and incubated at 26°C for several days. The fungal mycelium was then separated and transferred onto a substratum with glycosil-potato substrate (PDA). After the pure cultures had been isolated, macroscopic and microscopic observations were carried out to determine the species and the types of fungi in accordance with the studies by Domsch (1980) and Litwinow (1967).

The results obtained were then statistically processed, using variance analysis and $LSD_{0.05}$ further to Tukey's test.

RESULTS AND DISCUSSION

The percentage of alfalfa seeds germinating normally was relatively low, *ie* about 78%. No significant differences in the share of seeds germinating normally after irradiation with laser rays were found (Table 1). Even though an increase in seed germination was noted after this treatment, the differences were statistically insignificant. In contrast, the percentage of seeds germinating abnormally decreased significantly in comparison with the control under the influence of the following doses of surface power density of divergent laser light beam in the irradiation plane: R 3x3; R 3x5; R 6x3; R 6x5. The highest share of seeds germinating normally and the lowest share of seeds germinating abnormally were noted in the combination with the Funaben T seed dressing. The remaining two dressings had a similar influence on the germinating seeds but their effect was a little lower. The total of seeds which germinated was very high (above 97%) and did not differ significantly under the influence of the factors introduced.

The percentage of hard seeds and seeds infected with disease was very low, *ie* about 2 and 0.7%, respectively (Table 2). Under the influence of laser treatment, the percentage content of hard seeds decreased significantly when compared with the control, irrespective of the power dose applied. Similar relations were also found for those seeds infected with disease but particularly in the case of the doses of the surface power densities viz:- R 6x1; R 6x3 and R 6x5. All three seed dressings significantly decreased both the percentage of hard seeds and of seeds infected with disease. In this last case, the Funaben T and Sarfun T 65 DS dressings completely destroyed the fungal pathogens infecting the seed covers. Similar results were reported by Narkiewicz-Jodko (1990) and Bieniaszewski (2001) in leguminous plant seeds. The total of non-germinating seeds varied significantly and ranged from 2.3% (seed dressings) through 2.8-2.9% (laser treatment) to 5% (control). It should be stressed here that the American variety 'Legend' is characterized by its considerably lower share of hard seeds and seeds infected with disease in comparison with the Polish varieties of hybrid alfalfa (Skrzyniarz, 1987).

The highest number of potentially phyto-pathogenic fungi of the *Alternaria* type and the saprophytic fungi of the *Penicillium* type were isolated on the seed cover (Table 3). Laser light with a dose and power of the divergent beam of R

T a b l e 1. The influence of laser treatment and dressing of alfalfa sowing material on seed germination

Factors	% of seeds		Total of seeds germinating (%)
	germinating normally	germinating abnormally	
Irradiation dose and multiplication:			
R 0	73.0	22.0	95.0
R 3x1	76.5	21.0	97.5
R 3x3	77.8	19.0	96.8
R 3x5	79.0	18.0	97.0
Mean	77.8	19.3	97.1
R 6x1	76.0	21.0	97.0
R 6x3	80.0	17.8	97.8
R 6x5	79.4	17.4	96.8
Mean	78.5	18.7	97.2
Seed dressings:			
Funaben T	80.4	17.4	97.8
Sarfun T 65 DS	78.0	20.5	98.5
Super-Homai 70 DS	77.8	19.0	96.8
Mean	78.7	19.0	97.7
$LSD_{0.05}$	-	1.98	-

T a b l e 2. The influence of laser treatment and the dressing of alfalfa seed on the share of hard seeds and seeds infected with diseases

Factors	% of seeds		Sum of non-germinating seeds (%)
	hard seeds	seeds infected with diseases	
Dose and multiplication of irradiation:			
R 0	3.8	1.2	5.0
R 3x1	1.5	1.0	2.5
R 3x3	2.0	1.2	3.2
R 3x5	2.0	1.0	3.0
Mean	1.83	1.1	2.9
R 6x1	2.3	0.7	3.0
R 6x3	1.7	0.5	2.2
R 6x5	2.5	0.7	3.2
Mean	2.2	0.6	2.8
Seed dressings:	2.2	0.0	2.2
Funaben T	1.5	0.0	1.5
Sarfun T 65 DS	2.2	1.0	3.2
Super-Homai 70 DS			
Mean	2.0	0.3	2.3
LSD $_{0.05}$	0.19	0.09	0.3

T a b l e 3. The influence of laser treatment and the seed dressing on the number of fungi isolated from the seeds of alfalfa sowing material

Factors	Strains most frequently occurring		Number of strains isolated
	Alternaria	*Penicillium*	
Dose and multiplication of irradiation:			
R 0	3.0	1.0	5.0
R 3x1	1.0	3.0	7.0
R 3x3	4.0	1.0	8.0
R 3x5	4.0	1.0	7.0
Mean	3.0	1.7	7.3
R 6x1	4.0	2.0	7.0
R 6x3	1.0	0.0	2.0
R 6x5	1.0	0.0	3.0
Mean	2.0	0.7	4.0
Seed dressings:	0.0	0.0	0.0
Funaben T	0.0	0.0	0.0
Sarfun T 65 DS	2.0	0.0	7.0
Super-Homai 70 DS			
Mean	0.7	0.0	2.3
LSD $_{0.05}$	0.26	0.1	0.41

T a b l e 4. The influence of the multiplication of laser irradiation doses on the germination and health status of alfalfa seed

Specification	Multiplication of irradiation			Mean
	1	3	5	
1. Percentage of seeds:				
germinating normally	76.2	78.9	79.2	78.1
germinating abnormally	21.0	18.4	17.7	19.0
hard	1.9	1.8	2.2	2.0
infected with disease	0.8	0.8	0.8	0.8
2. Most frequently occurring strains:				
Alternaria	2.5	2.5	2.5	2.5
Penicillium	2.5	0.5	0.5	1.2
3. Total number of strains isolated	7.0	5.0	5.0	5.7

3x3; R 3x5 and R 6x1 significantly stimulated the growth of fungi of the *Alternaria* type, whereas laser light with a surface power density of R 3x1; R 6x3 and R 6x5 significantly decreased the number of these strains. Fungi of the *Penicillium* type behaved differently. A single irradiation treatment with a dose of 3 mW cm^{-2} resulted in a significant increase in the number of these strains compared to the control. A double dose of power with a single irradiation also resulted in a significant growth in fungi numbers of the *Penicillium* type, whereas a laser light beam with a surface power density of R 6x3 and R 6x5 destroyed them completely. The same effect was observed in the case of this fungal species when all three seed dressings were applied. On the other hand, fungi of the *Alternaria* type were completely destroyed by such dressings as the Funaben T and Sarfun T 65 DS. The most frequent *Alternaria* type was *Alternaria alternata*.

Multiplication of irradiation with laser light did not significantly influence the percentage of seeds germinating normally, seeds infected with disease, or the number of fungal strains of the *Alternaria* type (Table 4). The three- and five fold irradiations decreased the percentage share of those seeds germinating abnormally and those seeds infected with fungi of the *Penicillium* strain as well as the total number of strains isolated. The 'Legend' alfalfa seed variety is distinguished by its high sowing quality as specified by international regulations (ISTA, 1999).

CONCLUSIONS

1. The laser treatment of alfalfa seed did not cause any significant variation in the share of normally germinating seeds. A similar lack of effect was noted in the case of the seed dressing treatments. The percentage of abnormally germinating seeds decreased significantly under the influence of the irradiation doses and multiplications applied with the exception of the R 3x1 and R 6x1 doses and some seed dressings (Funaben T and Super-Homai 70 DS).

2. Laser treatment had a significant influence on the decrease in the content of hard seeds irrespective of the divergent laser beam power or its multiplied use. A similar effect was found after all three dressings had been applied. A significant decrease in the percentage of seeds infected with disease was noted in the combination with seed dressing and laser irradiation with a double power dose.

3. The amount of fungi of the *Alternaria* type increased significantly under the influence of the following doses of surface power density of the divergent laser light beam: R 3x3; R 3x5; R 6x1, the most frequent type being *Alternaria alternata*. Fungi of the *Penicillium* type infecting the seed covers were completely destroyed by the laser light with a surface power density and increase of: R 6x3 and R 6x5. The application of seed dressings completely destroyed fungi of the *Penicillium* type and most of the *Alternaria* type (except for Super-Homai 70 DS dressing).

4. The alfalfa seed studied (var. 'Legend') was characterized by the highest sowing quality of its seeds.

REFERENCES

Bieniaszewski T., 2001. Some agrotechnical factors determining the growth wholesomeness and yielding of yellow lupine varieties (in Polish). WUW-M Olsztyn, 51.

Domsch K.H., 1980. Compendium of Soil Fungi. Academic Press, London.

Duczmal K.W. and Tucholska H., 2000. Seed Production Science (in Polish). PWRiL, Poznań.

ISTA, **1999.** International rules for seed testing. Seed Sci. Technol., 24, supplement.

Koper R. and Dygała Z., 1994. Apparatus for pre-sowing seed treatment with laser irradiation (in Polish). Patent RP, No. 162598.

Litwinow M.A., 1967. Microscopic Fungi Guide (in Russian). Nauka, Leningrad.

Martin I.P., 1950. Use of acid rose Bengal and streptomycin in the plate method of estimating soil fungi. Soil. Sci., 19, 215.

Narkiewicz-Jodko M., 1990. The influence of seed dressing on sowing value and microflora during storage of pea seeds (in Polish). Biul. IHAR, 173-174.

Podleśny J., 2002. Studies on the influence of laser light on seeds, growth, development and yields of white lupine *(Lupinus albus* L.) (in Polish). Monografie i rozprawy naukowe, IUNG, Puławy, 3.

Podleśny J., 1997. Influence of the pre-sowing seed irradiation with laser light on the formation of morphological feature and faba bean yields (in Polish). Zesz. Probl. Post. Nauk Roln., 446, 435-440.

Skrzyniarz H., 1987. Influence of seed quality, sowing depth and density on growth and yielding of alfalfa.PartI: Effect of sowing density and hardness of seeds (inPolish). Pam. Pul., 90, 159-169.