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Foreword

The International Sunflower Association (ISA) and the Argentine Sunflower Association (ASAGIR) are pleased to present this guide to the 18th International Sunflower Conference.

At the time the main objectives for the meeting were defined, organizers aimed to provide a forum for the international sunflower research community with interest in any aspect of science and technology relating to the crop (in its oil-seed and confectionery variants) that would allow all involved to:

- Update knowledge in all fields of sunflower research since the previous conference held at Córdoba, Spain, June 2008;
- Review recent technological advances in sunflower production and identify knowledge gaps that require attention;
- Analyze the status and expectations for current and prospective demands for sunflower products;
- Provide a venue for workshops and special-interest meetings focusing on unresolved research, market, and production issues;
- Provide new generations with an opportunity to interact with global leaders in sunflower research.

The local Program Committee, with the help of the International Steering Committee, has developed a program covering the whole spectrum of relevant topics from genes and genomics through to field agronomy, crop protection, and industry and market issues. The program comprises 14 plenary and 13 invited presentations, 14 short oral presentations, an exhibition of 160 posters that can be visited during each of the first three days of the meeting. In addition, there will be three associated workshops (Bird Damage, Breeding, International Sunflower Genome Initiative), a special-interest presentation of the Global Crop Diversity Trust, and facilities will be available on request for small groups who wish to discuss business or scientific topics.

On the last day of the meeting, the Conference Field Day will be held at the joint INTA-Universidad de Mar del Plata facility in Balcarce. This time the traditional Conference demonstration plots of hybrids from International Sunflower Association member countries and from the host country will be complemented by a broad range of demonstrations of production and management techniques, as well as demonstrations of research techniques in current use by Argentine sunflower research teams.

This Conference has been made possible by the work of many people, by the support of sponsors from both the public and the private sector (sponsors are recognized on the back covers of this guide) and last, but certainly by no means least, those responsible for the lectures, short oral presentations, posters, associated workshops and special interest meetings, and field and laboratory demonstrations that make up the rich and varied bill of fare for this Conference, as reflected in this guide. The Organizing Committee extends their heartfelt thanks to all these individuals and organizations.

ISA and ASAGIR trust that this guide will enable all attendees to have an interesting and fruitful 18th International Sunflower Conference.

Welcome

It has been 27 years since the 11th International Sunflower Conference was held in Mar del Plata, Argentina, March 10-13, 1985. Since then, very many things have changed in the world of sunflower science, technology, and crop production and management. As the global sunflower community reconvenes once again in the same city, its members will have the opportunity to review progress in the last four years, which has been substantial in many areas.

Mar del Plata, a vibrant city located by the sea, with a fishing port, good restaurants, an unusually good choice of golf courses, and kilometers of sandy beaches, together with Balcarce, provide excellent venues for the Conference lectures and Field Day, and will allow attendees to appreciate a unique combination of seas, hills and Pampas. It is a great pleasure for the Organizing Committee to be able to host attendees to this meeting, which we hope will be both enjoyable and fruitful.

Welcome to Argentina, to Mar del Plata and Balcarce, and to the 18th International Sunflower Conference.

| 02-VC-20 |

Influence of substrate, chemical treatment and length of storage on sunflower seed germination

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ABSTRACT

- For determination of sunflower seed quality several substrates are in use: sand, soil, compost and filter paper where sand is most commonly used. Sand enables good contact of seeds and substrate and decreases possibility of contamination with pathogens. In addition, sand could be reused after sterilisation while other substrates are recommended for one use, which makes them more costly. For that reason, soil, compost and filter paper are not so frequently used. They find their place in research and comparative studies.
- Aim of this research was to determine if there is interaction between type of substrate and seed germination especially in case of seed protected with pesticides and stored in storage facilities.
- Research was conducted using hybrids NS-H-111 and Sremac, created in Institute of Field and Vegetable Crops. Seed germination was determined in laboratory conditions using standardized test, on hundred seed sample in four replications and temperature of 25°C and relative humidity of 95% with ten-day incubation period. Seed was germinated in sand, compost and filter paper. Study was done on seed treated with products containing metalaxyl, fludioxonyl, thiametoxam and imidacloprid as active ingredient, nine months before research started. Untreated seed was used as a control. Experimental design of trial was three-factorial completely randomized design and results were compared using analysis of variance.
- Influence of substratum, chemical treatment and lenght of storage on germination of hybrids NS-H-111 and Sremac was statistically highly significant. Interactions seedling substratum x length of storage and chemical treatment x length of storage was highly significant for both hybrids while, while difference for the interaction seedling substratum x chemical treatment was significant only for hybrid NS-H-111.
- Importance of this research is in broadening knowledge of interactions of different factors influencing seed vitality during storage as well as study the efficiency in using different modification of germination test in order to get reliable results.

Key words: Chemical treatment - seed germination - storage length - substrate

INTRODUCTION

Sunflower is probably the most important crop for oil production and prefered by consumers due to its high content of polyunsaturated fatty acids (Maiti, 2009). As a result of the high consumption of oil, sunflower is grown on area of over 23 million hectares in the world (http://faostat.fao.org). Therefore, large quantities of high quality hybrid seed are required.

Parameters of seed quality, germination energy, germination and emergence under field conditions, are crucial and directly determine the number of plants per hectare as one of three basic components of yield. Furthermore, the seed quality affects the rate and uniformity of germination and growth rate of plants (Crnobarac, 1992). To attain high yield of hybrids, application of good field practice, irrigation and use of high quality seed is recommended (Milosević et al., 1996). With the production of high quality seeds we create basic preconditions for maximal use of cultivar's genetic potential (Šimić et al., 2009).

To control the plant diseases and pests, use of pesticides as seed treatment has become a necessary and accepted practice (Jogi et al., 2010). The sunflower hybrid seed not planted in the first year remains to be planted in the second or even the third year after production (Mrđa et al., 2011). Basically more seeds are produced than what is needed and part of the seeds produced is kept as a stock (Šimić et al., 2009). Seed deterioration is a serious problem in countries where seed is stored in places without adequate control of humidity and temperate (Mohammadi et al., 2011). Seed storage length affects seed germination (Bonner, 1990; Colbach et al., 2002), because the seed vigor during storage progressively decreases, which reflects negatively on germination (Kashypa et al., 1994), and later on growth and development (Mrđa et al., 2009).

Type of substratum is one of the factors that also have influence on seed germination (Nijenstein and Ester, 1998; Tonkin, 1987), because the percentage of germinated seed is increasing with increasing of absorption capacity of substratum (Mrđa et al., 2010). The main type of substratum for testing sunflower seed is sand, because sand provides good contact of seed and substratum and minimizing the possibility of infection with pathogens. Unlike sand, which can be re-sterilized, compost and filter paper are recommended for single use. Therefore, these substratum are less commonly used for testing seed germination. They are most commonly use for comparative testing or research purposes.

Aim of this research was to determine if there is interaction between type of substrate and seed germination especially in case of seed protected with pesticides and stored in storage facilities.

MATERIALS AND METHODS

Research was conducted on commercial sunflower seed, NS-H-111 and Sremac, developed at the Institute of Field and Vegetable Crops in Novi Sad. Seed germination was determined in laboratory using standardized test, on hundred seed sample in four replications and temperature of 25°C and relative humidity of 95% with ten-day incubation period (ISTA, 2007). Seed was germinated in sand, compost and filter paper.

Seed used in research was treated with products for seed treatment with active ingredients metalaxyl, fludioxonyl, thiametoxam and imidacloprid, and control was untreated seed. Products with fludioxonyl and metalaxyl as a.i. were applied in the quantity of 300ml/100kg of seed, and those with thiametoxam and imidacloprid as a.i. in the quantity of 1000ml/100kg of seed.

Treated seed was kept stored for nine months in paper bags in stock used for storage of commercial seed. Testing was carried out after treatment, after three, six and nine months of treatment. The following combinations were chosen in research:

- Control (untreated seed),
- Fludioxonyl + metalaxyl (F+M),
- Fludioxonyl + metalaxyl + thiametoxam (F+M+T) and
- Fludioxonyl + metalaxyl + imidacloprid (F+M+I).

The obtained results were statistically processed using three-way *ANOVA* for completely randomized design (A factor – seedling substratum, B factor – chemical treatment, C factor – length of storage using software *STATISTIKA 10*. Results were compared using Least Significant Difference (LSD) post-hoc test, on 1 and 5 % level of significance (Mead et al., 1996).

RESULTS

F-test for main effects of seed germination of hybrid NS-H-111 was highly significant. In addition to this, all interactions of studied factors were highly significant (Table 1).

Table 1. *F-value* from *ANOVA* for effect of seedling substratum (S), chemical treatment (C), length of storage (L) and interactions seedling substratum x chemical treatment (SC), seedling substratum x length of storage (SL), chemical treatment x length of storage (CL) and seedling substratum x chemical treatment x length of storage (SCL) on hybrid NS-H-111 seed germination

S	C	L	SC	SL	CL	SCL
33.21**	52.66**	53.86**	12.46**	6.38**	22.10**	2.73**

**Significant at 1% level of probability.

The results of hybrid NS-H-111 germination rate on different seedling substratum after various periods of germination are presented in Table 2. On average, seed germination was highest if the compost is used as a substratum (94.62%). Value obtained for germination on filter paper (90.64%) was significantly lower than the value obtained for germination in sand (2.92% difference) and compost (3.98% difference). The difference between sand and compost (1.06%) was significant.

Germination of hybrid NS-H-111seed treated with insecticides was significantly lower compared to germination of control (94.94%) and treatment with fungicides (95.02%). The lowest value of germination had a combination *fludioxonyl + metalaxyl + imidacloprid* (88.62%) and this value was significantly highly lowest compared to other tested combinations. Moreover, difference between control and treatment with fungicides was not significant.

Testing of seed germination at different periods after treatment, on average, showed statistically significant effects. Value obtained after six months of storage (95.35%) was significantly higher than after germination tests immediately after treatment and after three months of storage (93.96%), and highly significantly higher than the value after nine months of storage (88.50%). Germination obtained after nine months of storage was significantly lower compared to the first two tests.

Table 1. Seed germination of hybrid NS-H-111 (%)

Seedling substratum (S)	Chemical treatment (C)	Length of storage – months after treatment (L)				Average (SΔC)	Average (S)
		0	3	6	9		
Filter paper	K	95.75	92.75	97.75	97.00	95.81	90.64
	F + M	96.00	92.25	95.50	91.75	93.88	
	F + M + T	95.00	83.25	93.75	88.00	90.00	
	F + M + I	85.75	88.75	90.75	66.25	82.88	
	Mean (SΔL)	93.12	89.25	94.44	85.75		
Sand	K	93.75	96.25	96.25	90.25	94.12	93.56
	F + M	92.25	96.50	97.50	92.50	94.69	
	F + M + T	94.75	93.25	97.00	89.75	93.69	
	F + M + I	94.00	96.25	95.25	81.50	91.75	
	Mean (SΔL)	93.69	95.56	96.50	88.50		
Compost	K	95.25	96.75	94.00	93.50	94.88	94.62
	F + M	96.00	97.25	95.50	97.25	96.50	
	F + M + T	94.25	98.25	96.25	94.75	95.88	
	F + M + I	94.75	96.00	94.75	79.50	91.25	
	Mean (SΔL)	95.06	97.06	95.12	91.25	Mean (C)	
Mean (CΔL)	K	94.92	95.25	96.00	93.58	94.94	92.94
	F + M	94.75	95.33	96.17	93.83	95.02	
	F + M + T	94.67	91.58	95.67	90.83	93.19	
	F + M + I	91.50	93.67	93.58	75.75	88.62	
Mean (L)		93.96	93.96	95.35	88.50		

	S	C	L	S x C	S x L	C x L	S x C x L
$LSD_{0,05}$	1.00	1.16	1.16	2.00	2.00	2.31	4.00
$LSD_{0,01}$	1.32	1.53	1.53	2.64	2.64	3.05	5.29

K = control (untreated seed)
F + M = fludioxonil + metalaxyl
F + M + T = fludioxonil + metalaxyl + thiamethoxam
F + M + I = fludioxonil + metalaxyl + imidacloprid

Results of analysis of variance for interaction are somewhat similar to the effects of main factors. For all three substratum, the lowest value of germination had seed treated with *fludioxonil + metalaxyl + imidacloprid* combination. Germination on filter paper was significantly different between treatments *fludioxonil + metalaxyl + thiamethoxam* (90.00%) and control (5.81% difference) and *fludioxonil + metalaxyl* treatment (3.88% difference). Interaction seedling substratum x length of storage indicates statistically significant germination decrease after nine months of storage, for all substratum, while interaction chemical treatment x length of storage had minimal germination in case of seed treated with *fludioxonil + metalaxyl + imidacloprid* combination after nine months of storage.

The effect of seedling substratum, chemical treatment and length of storage on seed germination of hybrid Sremac, based on the F-test for main effects was significant. Interactions seedling substratum x length of storage and chemical treatment x length of storage was highly significant, while difference for the interaction seedling substratum x chemical treatment was not significant (Table 3).

Table 4 shows the results of hybrid Sremac seed germination in different seedling substratum and for various periods of testing. On average, significantly highest value of seed germination was on filter paper (96.17%). Value obtained for germination in sand (94.88%) was significantly higher compared to germination in compost (0.77% difference).

Table 3. *F-value* from *ANOVA* for effect of seedling substratum (S), chemical treatment (C), length of storage (L) and interactions seedling substratum x chemical treatment (SC), seedling substratum x length of storage (SL), chemical treatment x length of storage (CL) and seedling substratum x chemical treatment x length of storage (SCL) on hybrid Sremac seed germination

S	C	L	SC	SL	CL	SCL
15.52^{**}	24.52^{**}	26.88^{**}	1.72^{ns}	11.06^{**}	5.38^{**}	3.50^{**}

**Significant at 1% level of probability and ^{ns} not significant.

Germination of hybrid Sremac was lowest in treatments with insecticides. However, significant differences were detected only for *fludioxonil + metalaxyl + imidacloprid* treatment (92.81%), whereas differences between *fludioxonil + metalaxyl + thiamethoxam* and *fludioxonil + metalaxyl* treatments and control were not significant. Moreover, differences between *fludioxonil + metalaxyl* treatment and control were not significant.

Table 4. Seed germination of hybrid Sremac (%)

Seedling substratum (S)	Chemical treatment (C)	Length of storage – months after treatment (L)				Average (SΔC)	Average (S)
		0	3	6	9		
Filter paper	K	96.50	93.50	97.50	97.50	96.25	96.17
	F + M	96.25	97.25	97.50	97.50	97.12	
	F + M + T	98.75	94.75	97.25	97.75	97.12	
	F + M + I	95.75	92.25	95.50	93.25	94.19	
	Mean (SΔL)	96.81	94.44	96.94	96.50		
Sand	K	95.50	96.75	96.75	93.50	95.62	94.88
	F + M	95.75	96.50	98.00	92.75	95.75	
	F + M + T	92.75	97.50	96.50	92.50	94.81	
	F + M + I	91.25	95.25	96.50	90.25	93.31	
	Mean (SΔL)	93.81	96.50	96.94	92.25		
Compost	K	95.25	95.50	97.50	93.75	95.50	94.11
	F + M	95.75	95.75	95.75	94.50	95.44	
	F + M + T	93.75	94.50	96.75	93.25	94.56	
	F + M + I	94.50	91.75	97.50	80.00	90.94	
	Mean (SΔL)	94.81	94.38	96.88	90.38	Mean (C)	
Mean (CΔL)	K	95.75	95.25	97.25	94.92	95.79	95.05
	F + M	95.92	96.50	97.08	94.92	96.10	
	F + M + T	95.08	95.58	96.83	94.50	95.50	
	F + M + I	93.83	93.08	96.50	87.83	92.81	
Mean (L)		95.15	95.10	96.92	93.04		

S	C	L	S x C	S x L	C x L	S x C x L

$LSD_{0,05}$	0.74	0.85	0.85	1.48	1.48	1.71	2.96
$LSD_{0,01}$	0.98	1.13	1.13	1.95	1.95	2.26	3.91

K = control (untreated seed)
F + M = fludioxonil + metalaxyl
F + M + T = fludioxonil + metalaxyl + thiamethoxam
F + M + I = fludioxonil + metalaxyl + imidacloprid

Length of storage had a significant influence on the seed germination of tested hybrid. Significantly highest value of seed germination had seed kept six months (96.92%). Furthermore, there was a decline of germination after nine months of storage (93.04%). Value obtained in this test was highly significantly lowest. The difference between the first two tests was significant.

Results for interaction seedling substratum x chemical treatment demonstrate that effect of chemical treatment depends on seedling substratum. *Fludioxonil + metalaxyl +imidacloprid* combination had negative effect on seed germination in all three testing substratum. The obtained values were significantly highly lower compared to other treatments, for both compost and filter paper, and significantly lower germination in the sand. Combination with other formulations of pesticides (*fludioxonil + metalaxyl + thiamethoxam*) has also negative effect on seed germination. The obtained values were lower compared to control and treatment with fungicides, but not significantly. Interaction seedling substratum x length of storage indicate that there was significant decrease of seed germination after nine months of storage where sand and compost were used as substratum, while the germination on filter paper had significantly lowest value after the three months of storage. Interaction indicates that the highly significantly lowest germination had seed treated with *fludioxonil + metalaxyl + imidacloprid* combination, for all lengths of storage, except for seed kept for six months, where no significant difference was detected.

DISCUSSION

According to the results of this research the effect of seedling substratum, chemical treatment and length of storage on seed germination of hybrids NS-H-111 and Sremac was significant.

Differences in seed germination on different type of seedling substratum are correlated with adsorption capacity (Nijenstein and Ester, 1998). These authors concluded that percentage of germination increases with usage of substratum with high adsorption capacity. Other authors gained similar findings for various crops (Fuchs and Weigand, 1985; Steiner and Fuchs, 1987; Tonkin, 1987). However, Perez et al. (1999) in their study did not find significant differences in the use of different substratum on seed germination. Effect of the substratum on seed germination is influenced by genotype and therefore more than one genotype should be included in this type of research aiming to minimize effect of this factor. On the other hand, for each genotype optimal substratum should be chosen to provide reliable results.

This study showed negative effect of chemical treatment, particularly combinations with insecticides, on seed germination. Sajjan et al. (2010) in his work indicate a significant effect of imidacloprid on germination. Kuhar et al. (2002) report cases of decreased germination of sweet corn due to the influence of imidacloprid, and Kashypa et al. (1994) showed that the used of insecticides extended period of germination and reduce seed vigor. Opposite to these results Grisi et al. (2009) concluded that there was no effect of treatment with fungicides, insecticides and their associations in sunflower seed.

Wang et al. (2009) came to the conclusion that seed storage length have significant effect on germination. Increase in sugar beet seed germination energy and germination after a period of six months after harvest is reported by Rajić et al. (2005). In this research reason for increase of seed germination is probably due to end of seed dormancy. Marjanović-Jeromela et al. (2008) examined the impact of treatment with fungicides and insecticides on rapeseed seed quality after 21 months storage and determined decrease in germination, germination energy and seed vigour. Stanković and Medić (1997) concluded that germination energy and germination of sunflower and maize seed treated with insecticides was reduced in all treatments, although not significant in all cases. However, all insecticides have negative effect after one-year storage. Contrary to the results of this research, Ghasemnezhad and Honermeier (2009) found no significant influence of the storage length on seed germination.

This research pointed on high dependence of genotype and substratum. Further research should include more genotypes which would help in selection of the substratum most suitable for determination of seed germination for majority of genotypes. Furthermore, this research confirmed that choice of optimal combination of pesticides for seed treatment is extremely important for maintaining seed quality during storage. Hence, apart from choice of pesticides, decision of time for treating seed should be adjust to minimize loss in seed vigour.

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