Possibility of Using Seed Treatment to Suppress Seed-Borne Diseases in Poppy

Tomáš SPITZER[1], Dagmar SPITZEROVÁ[1], Pavel MATUŠINSKÝ[1] and Jan KAZDA[2]

[1]Agrotest fyto, Ltd., Kroměříž, Czech Republic; [2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic

ABSTRACT

Spitzer T., Spitzerová D., Matušinský P., Kazda J. (2014): **Possibility of using seed treatment to suppress seed-borne diseases in poppy**. Plant Protect Sci., **50**: 78–83.

In experiments using Petri dishes in the laboratory and pots in a greenhouse and climate chamber, we examined the influence of seed treatment on emergence of poppy. Four types of fungi (*Alternaria* spp., *Dendryphion penicillatum*, *Fusarium* spp., and *Penicillium* spp.) were detected on poppy seeds, with the highest infection rate being 72% for *D. penicillatum*. Surface disinfection decreased infection rate chiefly in *D. penicillatum* (by 32%) and in *Alternaria* spp. (by 16%). Seed treatment increased emergence by 9–10% in laboratory experiments but by only 0–6% in greenhouse experiments. Temperature plays an important role in emergence. In climate chamber experiments at a stable temperature of 12°C, the seed treatments increased emergence by 8–16%.

Keywords: *Alternaria* spp.; *Dendryphion penicillatum*; *Fusarium* spp.; *Penicillium* spp.; iprodione; carboxin; thiram; tebuconazole

Poppy is a traditional crop grown in the Czech Republic. Its planted areas and yields vary greatly by individual year, and it is one of the highest-risk crops to raise. A key aspect of growing poppy successfully is to establish a homogeneous stand. Such a stand can only be achieved when the sown seeds emerge uniformly within a given time period (Kamkar *et al.* 2012). Achieving such emergence in poppy is very difficult and depends upon many factors, such as sowing depth, soil moisture and, very importantly, the elimination of detrimental effects from seed-borne diseases.

Among the most damaging seed-borne pathogens are *Dendryphion penicillatum* (Corda) *Fr.* and *Pleospora papaveracea* (de Not.) *Sacc.* (syn. *Pleospora calvescens*). These two fungi were formerly described as the asexual and sexual states of a single fungus, but Farr and O'Neill (2000) discovered that the anamorph of *P. papaveracea* is a *Dendryphiella*-like fungus and that *D. penicillatum* lacks a known sexual state.

The fungi, which have been isolated from seed samples originating in Iran, Colombia, Venezuela, Sweden, India, and the USA, cause dieback in germinating poppy plants (O'Neill *et al.* 2000).

Another important pathogen of poppy, *Peronospora arborescens* (Berkeley) de Bary, also can be seed-borne. In its case, seeds are infected within infected capsules in secondarily infected plants (Montes-Borrego *et al.* 2009). Landa *et al.* (2007) determined that severe downy mildew diseases of opium poppy (*Papaver somniferum* L.) can be caused by *P. arborescens* and *P. cristata*, but differentiating between the two pathogens is difficult because they share morphological features and a similar host range. These authors also determined that the pathogenic fungi are seed-borne and that seed from stands with high incidence of the disease is also highly infected.

The aims of this study were to determine whether selected active ingredients used in seed treatments would improve the emergence of poppy plants by eliminating seed-borne fungal pathogens, whether the substances used would negatively influence the emergence of poppy, and whether temperature dur-

Supported by Institutional support provided for long-term development of research organisation, Decision of the Ministry of Agriculture of the Czech Republic, No. RO0211 of 28 February.

ing poppy germination affects the investigated fungal pathogens. The ultimate aim, therefore, was to determine the effectiveness of such seed treatment.

MATERIAL AND METHODS

Poppy seeds of the Marathon cultivar harvested from a common stand not treated with fungicides were used in the experiments.

BD Sabouraud Glucose Agar (Becton, Dickinson and Company, Franklin Lakes, USA), which is used for isolating and cultivating mould in the medical field, was utilised for determining the species representation of phytopathogenic fungi on poppy seeds used in the experiments, as well as the infection rates of individual seeds. A high glucose concentration is advantageous for the growth of fungi, while it prevents poppy seeds from taking up water and thus from germinating. Fifty seeds were planted on Petri dishes without surface disinfection and the same number after double surface disinfection using SAVO disinfecting agent (Bochemie, Bohumín, Czech Republic), which contains 5% sodium hypochlorite (NaClO), in order to determine the fungal species spectrum on the seeds and whether certain fungi grow into the seeds or only adhere to the surface.

The influence of seed treatment was determined in the three following types of experiments:

Laboratory tests in Petri dishes (10 cm diameter) on wet filter paper. Three series of identical tests were performed, always planting 100 seeds in four repetitions for each experimental variant on Whatman No. 1 filter paper (GE Healthcare Life Sciences). The experiments were conducted at the temperature of 22°C. The results were evaluated according to International Seed Testing Association (2011) methodology 5 and 8 days after planting. After 5 days, the seeds were divided into two categories for evaluation: germinated (plants with formed cotyledons) and not germinated. In the second evaluation after 8 days, three categories were formed: germinated, not germinated, and underdeveloped plants. Underdeveloped plants were considered to be those which had markedly fallen behind in their development or which had not formed a root. In the control sample, plants which formed a root and a base of the cotyledons, but were very quickly destroyed by fungal pathogens, and mainly by the species *D. penicillatum*, were also considered as underdeveloped.

Greenhouse experiments in small (10 × 10 cm) pots. Fifty poppy seeds were planted in each pot and these were placed in a greenhouse at the temperature of 18–20°C. Each variant was planted in four repetitions. Potting soil type B was used, which is a mixture of 990.5 l transitory and marsh peat + 2 l of chemical fertiliser (brand Cererit, 8:13:11) + 7.5 l $CaCO_3$ per kilolitre. Only one evaluation was performed, that being 14 days after planting, in the phase when the poppy plants were forming cotyledons.

Table 1. A list of applied active ingredients

Active ingredient	Content	Note
Iprodione	50%	registered for poppy
Carboxin + thiram	200 g + 200 g/l	not registered for poppy
Tebuconazole + thiram	15 g + 500 g/l	not registered for poppy

Low-temperature tests. These were basically identical to the greenhouse tests, except that after planting the seeds in the pots were placed into a low-temperature chamber where a 12°C temperature was maintained and the light cycle was set to 12 h day and 12 h dark. Just one evaluation was performed, that being 14 days after planting, in the phase when the poppy plants were forming cotyledons (Table 1).

Statistical analysis. STATISTICA, Version 7.0 software package (StatSoft, Tulsa, USA) was used for statistical analysis by regression and Analysis of Variance (ANOVA).

RESULTS

Table 2 summarizes the infection rates and species spectrum for fungi detected on glucose agar among poppy seeds used in the experiments. Four types of fungi were detected: *Alternaria* spp., *Dendryphion penicillatum*, *Fusarium* spp., and *Penicillium* spp. At 72% (36 seeds infected out of 50), the infection rate by *D. penicillatum* could be considered high. The infection rate for *Penicillium* spp. was 40% (20 seeds out of 50), and for *Alternaria* spp. it was 44% (22 seeds out of 50). *Fusarium* spp. infection occurred at a low rate of 14% (7 seeds out of 50). In a number of cases, mixed infection by several species of fungi occurred in individual seeds. Only one seed was found to be wholly uninfected by fungal pathogens.

After surface disinfection of the seeds, their infection rates markedly decreased. This was especially the case in relation to *D. penicillatum* (40% final infection rate, reduction by 32 percentage points vs. seeds not disinfected) and *Alternaria* spp. (28% final

Table 2. Comparison of occurrence of fungal species on poppy seeds on osmotic agar without and with surface sterilisation

No. of tested seeds	No infection	*Alternaria* sp.	*D. penicillatum*	*Fusarium* sp.	*Penicillium* sp.
Control (no surface sterilisation)					
50	1	22	36	7	20
2 × Savo (NaClO 4.8% of active Cl)					
50	16	14	20	6	22

Numbers indicate the frequencies of individual fungi, some seeds were infected by more fungi species

infection rate, reduction by 16 percentage points). For *Penicillium* spp. and *Fusarium* spp. the final rates were identical to those for the variants without surface disinfection. The number of seeds with no fungal pathogens also increased, to sixteen.

The results of the laboratory experiments are summarised in Table 3 (evaluation after 5 days) and Table 4 (evaluation after 8 days). Little or no improvement in germination was observed when evaluating the treated seeds 5 days after planting, and the larger application rate of an active ingredient worked better only in the case of tebuconazole + thiram (improvement by 5% vs. the control). Evaluation after 8 days better indicated the seed treatment to have had a significant effect, especially because in the control samples part of the germinated plants had died by that time due to *D. penicillatum* infection while this did not occur in the treated variants. The reduction

Table 3. Results of laboratory assays (the first term of evaluation), of greenhouse experiment, and of cold test (at 12°C)

Variant	Dose	Experiments I–III			
		germinated (number)	% of control	non-germinated (number)	% of control
Laboratory assays[a]					
Control – untreated		65	100	35	
Iprodione	2000 g/t seeds	67	103	33	94
Carboxin + thiram	200 + 200 g/t seeds	66	102	34	97
Carboxin + thiram	400 + 400 g/t seeds	65	100	35	100
Tebuconazole + thiram	15 + 500 g /t seeds	65	100	35	100
Tebuconazole + thiram	30 + 1000 g /t seeds	68*	105	32*	91
Greenhouse experiments[a]					
Control – untreated		36	100	14	100
Iprodione	2000 g/t seeds	34	94	16	114
Carboxin + thiram	200 + 200 g/t seeds	36	100	14	100
Carboxin + thiram	400 + 400 g/t seeds	38*	106	12*	86
Tebuconazole + thiram	15 + 500 g /t seeds	37	103	13	93
Tebuconazole + thiram	30 + 1000 g /t seeds	36	100	14	100
Cold test[b]					
Control – untreated		37	100	13	100
Iprodione	2000 g/t seeds	40	108	10	77
Carboxin + thiram	200 + 200 g/t seeds	43**	116	7**	54
Carboxin + thiram	400 + 400 g/t seeds	42*	114	8*	62
Tebuconazole + thiram	15 + 500 g /t seeds	42*	114	8*	62
Tebuconazole + thiram	30 + 1000 g /t seeds	42*	114	8*	62

*significant at 0.05 probability level; ** significaqnt at 0.01 probability level; [a]100 poppy seeds were sown in four replications in each experiment; [b]50 poppy seeds were sown in four replications in each experiment

Table 4. Results of laboratory experiments – the second term of evaluation

Variant	Dose	Experiments I–III					
		germinated (number)	% of control	non-germinated (number)	% of control	dwarf (number)	% of control
Control – untreated		56	100	26	100	18	100
Iprodione	2000 g/t seeds	61	109	23	88	16	89
Carboxin + thiram	200 + 200 g/t seeds	61	109	23	88	16	89
Carboxin + thiram	400 + 400 g/t seeds	62	110	25	96	13*	72
Tebuconazole + thiram	15 + 500 g /t seeds	61	109	21	81	18	100
Tebuconazole + thiram	30 + 1000 g /t seeds	62	110	20	77*	18	100

*significant at 0.05 probability level; 100 poppy seeds were sown in four replications in each experiment

of plants relative to the control was 9 plants after 8 days, while in the treated variants it was only 4 plants. Therefore, the positive influence of seed treatment had a more marked effect in the range of 9–10% vs. the control. The effect was sensitive to application rate, as the higher-rate treatments attained greater numbers of plants in the germinated category. With tebuconazole + thiram at both application rates and carboxin + thiram at the lower rate, the number in the not germinated category was also markedly decreased. In the carboxin + thiram higher-rate variant and in the case of iprodione, the number of plants in the underdeveloped category was markedly decreased.

The results of the greenhouse experiments are summarised in Table 3 and comprise a composite of three identical greenhouse experiments performed in pots with potting soil at a temperature of 18–20°C. The results of these experiments are very similar to those observed in laboratory conditions when evaluated after 5 days. The number of germinated plants was little affected (in the range of 0–6% vs. the control) while the germination rate was even lower (by 6%) in the case of iprodione treatment. The reason for the differences in results of the laboratory test after 8 days and that of the greenhouse test after 14 days was the absence of visible infection by *D. penicillatum* in control variants for the poppy plants already emerged. Also, a higher total number of germinated plants was determined in experiments using potting soil in the greenhouse (72% germination in the case of the control) than in the laboratory tests (56% germination for the control).

DISCUSSION

Upon analyzing the range of fungal species occurring on the poppy seeds, it is noteworthy that *D. penicillatum* was dominant and that the contamination rate for seeds in a sample can reach very high values. *Alternaria* spp. are common representatives of saprophytic fungi occurring on dead plant tissues and their presence on poppy seeds was expected. Contamination probably had occurred during harvest by combine harvesters. The presence of the *Penicillium* genus is most probably caused by handling of the poppy seeds under laboratory conditions. Be that as it may, *Alternaria* spp. and *Penicillium* spp. have no great practical importance for the emergence and growth of poppy in field conditions (Mihaela *et al.* 2013).

Fusarium spp. presents quite another case, as these fungi are able to produce mycotoxins which can be important in poppy, particularly inasmuch as poppy seeds are intended for direct human consumption. The occurrence of these fungi was at very low levels, however, and it can be considered likely that this constituted a case of secondary contamination of seeds either from surrounding crops or from plant residues during harvest. During autumn 2011, Garibaldi *et al.* (2012) observed symptoms of a wilt disease on plants of *Papaver nudicaule* (Iceland poppy) in northern Italy. BLAST analysis showed an E-value of 0.0 and 100% identity with the internal transcribed spacer (ITS) sequence of *F. oxysporum* (HQ649820). To confirm pathogenicity, tests had been conducted by inoculating roots of poppy. Inoculated plants showed typical symptoms of Fusarium wilt after 10 days while non-inoculated plants remained healthy.

The use of repeated surface disinfection of poppy seeds should preclude germination and growth of mycelia from conidia adhering to the surface of the seeds and thus determine whether certain fungi are able also to grow into the seeds. After disinfection, the occurrence of *D. penicillatum* and *Alternaria* spp. on poppy seeds placed on osmotic agar markedly decreased while the infection rates for *Penicil-*

lium spp. and *Fusarium* spp. remained unchanged. This could be due to the fact that poppy seeds have a very rough and crenulated surface, and therefore it is possible that not all conidia were removed even after double disinfection of the surface. Selcuk *et al.* (2008) reported that using a low-pressure cold plasma system to achieve surface decontamination of cereal and legume seeds from *Aspergillus* spp. and *Penicillium* spp. fungi reduced the rate of seed contamination to less than 1% of its original, artificially contaminated rate.

The conidia size for the individual species of monitored fungi also can play an important part here. While the conidia of *D. penicillatum* and *Alternaria* spp. are large (40 × 9 nm and 100 × 10 nm, respectively), the conidia of *Fusarium* spp. in those species most commonly occurring under field conditions in the Czech Republic (*F. culmorum*, *F. graminearum,* and *F. avenaceum*) are slender and pointed (35–70 nm × 3–6 nm), and in *Penicillium* spp. they are round and very small (3.0–3.5 nm). The large and round conidia of *D. penicillatum* and *Alternaria* spp. can be more easily removed from the uneven surface of the seeds than can the thin and pointed conidia of *Fusarium* spp. and the small, round conidia of *Penicillium* spp. This also conforms to the increase in the number of seeds without infection after surface disinfection from 1 to 16 seeds. In order to definitively prove or disprove the theory that certain fungi grow into the interior of poppy seeds, it will be necessary to use other methods capable of proving, for example, the presence of the pathogen's DNA inside the embryo.

The influence of temperature on the infection rate of winter wheat by seed-borne common bunt (*Tilletia caries* (DC) Tul. and *Tilletia foetida* (Wall.) Liro) was monitored by Polisenska *et al.* (1998). They stated that higher infection occurred at lower soil temperatures after sowing without regard to sowing date. Liatukas and Ruzgas (2009) found the highest correlation between average daily temperature and infection rate for the occurrence of the bunt caused by *Tilletia caries* on wheat at 36 days after sowing and the lowest at 150 days after sowing.

Temperature plays an important role also in germination of poppy and for *D. penicillatum*. This fungus is capable of forming appressoria already 4 h after falling upon a leaf, and even at low temperatures of 7–13°C (Bailey *et al.* 2000). In poppy, temperature is a decisive factor for germination energy. At 10°C, seeds germinate within 5–6 days; at 18–20°C, germination is within 3–4 days.

Therefore, based upon the findings from the laboratory and greenhouse experiments, another experiment was designed utilising reduced temperature conditions. The results of three identical low-temperature tests are summarised in Tables 3. The number of germinated plants for the control was comparable to the number determined in the greenhouse tests. A marked and significant effect of the treatments was observed, however, as they increased the number of germinated plants by 8–16% vs. the control, while in greenhouse experiments the influence of the seed treatments was relatively low. It is clear that in the low-temperature conditions prevalent in early spring, when poppy is sown, the help of seed treatments can be important for improving poppy emergence. The same result might also be achieved, however, by using healthy seeds obtained while taking better care to ensure good health conditions in seed-growing areas.

Acknowledgement. The authors would like to thank English Editorial Services, s.r.o. for translation and revision of the manuscript.

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Received for publication October 15, 2012
Accepted after correction November 4, 2013

Corresponding author:

RNDr. Tomáš Spitzer, Ph.D., Agrotest fyto, s.r.o.., Kroměříž, Havlíčkova 2787, 76 701 Kroměříž, Česká republika;
E-mail: spitzer@vukrom.cz