Effect of fungicide seed treatment on rhizobial survival and nodulation of chickpea

S. Kyei-Boahen[1], F. L. Walley[2] and A. E. Slinkard[1]
[1]Crop Development Centre, [2]Department of Soil Science,
University of Saskatchewan, Saskatoon, SK

Introduction

Chickpea seeds are often treated with fungicides to prevent losses due to seed-borne pathogens and damping off. In addition, rhizobia are applied to the seeds to ensure effective nodulation and subsequent nitrogen fixation. Although reports are conflicting, several studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium*. Furthermore, it was demonstrated that different species and strains of the same species of *Rhizobium* differed in their sensitivity toward various fungicides. Therefore, the objectives of this study were to examine the effect of four commercial fungicides, Apron, Thiram (Arrest 75W®), Crown and Captan on 1) the survival of *Rhizobium ciceri* strain BCF 32 inoculated onto chickpea seeds and 2) nodulation, nitrogen fixation and dry matter production of chickpea in the growth chamber.

Materials and Methods

Seed sterilization and treatment

Seeds of desi chickpea were surface sterilized for 3 min in 70% alcohol followed by a 3 min treatment with 3% sodium hypochloride. The seeds were rinsed 6 times with sterile water, dried and treated separately with one of the four fungicides. The formulation, active ingredients and the rate of application of the fungicides used are listed in Table 1.

Table 1. List of fungicides used to treat chickpea seeds

Treatment	Formulation	Active ingredient	Rate (per kg seed)
Apron®-FL	Powder	28.35% metalaxy [N 2,6-dimethylpheny) –N-(methoxyacetyl) alanine	2.5g
Arrest 75W	Powder	50% thiram (tetramethyl thiuram disulfide) 20% carbathiin (5,6-dihydro 2-methyl-1,4 oxathiin-3-carboxanilide) 5% oxycarboxin (5,6-dihydro 2-methyl-1,4 oxathiin-3-carboxanilide 4, 4 -dioxide)	2.8g
Captan 50W	Powder	50% [N-tri-chloromethylthio-4-cyclohexene-1,2-dicarboximide	2.0g
Crown®	Liquid	92g l^{-1} carbathiin (5,6-dihydro 2-methyl.1,4oxathiin-3-carboxanilide) 58g l^{-1} thiabendazole [2-(1,3-thiozol-4-yl) benzimidazole]	18ml

The fungicide-treated seeds were then inoculated with peat inoculant containing *Rhizobium ciceri* strain BCF 32 (MicroBio RhizoGen Corp., Saskatoon) at the recommended rate, using gum arabic solution as sticker. The seeds were stored in glass bottles and kept in a refrigerator. Non-fungicide treated seeds were also inoculated and stored as before.

Rhizobial survival on treated seeds

At 4, 12, 24 and 48 h after inoculation, 40 seeds from each fungicide treatment were removed and and divided into four subsamples of 10 seeds. Each subsample was transferred into test tubes containing 10 ml sterile water. The test tubes were shaken vigorously to wash the inoculum off the seeds. One ml of the resultant suspension in each test tube was taken and serial dilution were made from each subsample. Then, 0.1 ml of each dilution was plated by the spread plate method on yeast extract-mannitol agar (YMA) (Vincent, 1970) containing Congo Red. The Congo Red aids in detecting contaminants. The plates were incubated at 26^{o}C and rhizobial colonies counted after 8 d. The experiment was repeated using the same fungicides and inoculant.

Growth chamber study of nodulation and dry matter yield

At each plating time, four seeds (i.e. sample from seeds stored in the survival experiment) from each treatment were planted into 2.5 L plastic pots containing a mixture of soil, sand and vermiculite in a 2:1:1 ratio (v/v). The pots were arranged in a randomized complete block design with four replicates. The plants were grown under 16-h daylength and a mean day and night temperature of about 25 and 18^{o}C respectively. After emergence, the plants in each pot were thinned to two after which a 25 ml solution, containing 20 mg of 10.5% ^{15}N enriched NH_4NO_3, was applied to the surface of the soil in each pot. Flax was also grown as reference crop for the assessment of nitrogen fixation by the ^{15}N-enrichment technique. The plants were watered when necessary with tap water and also with half-strength N-free Hoagland nutrient solution biweekly. Harvesting was done at the flowering and early pod filling stages to examine nodulation, nitrogen fixation and to determine dry matter yield.

Statistical analysis

Combined analyses for the survival experiments were performed using the General Linear Model software developed by SAS Institute. For the growth chamber study, data for the flowering and early pod filling stages were analyzed separately. Data for nitrogen fixation are not available to date.

Results and discussion

Fungicide treatment decreased the number of viable rhizobia on seeds but the effect was not significant for seeds treated with Crown (Fig. 1). Arrest, Apron and Captan reduced the numbers of rhizobia drastically after 4 h of initial fungicide-*Rhizobium* contact. Both Apron and Captan reduced the number of viable rhizobia during the first 12 h of contact. The deleterious effect of Arrest on rhizobial survival was not consistent. In general, the toxicity of the fungicides increased in the following order: Control < Crown < Arrest = Apron < Captan.

Rhizobia die rapidly on seeds following inoculation due to exposure to adverse environmental conditions, such as excessive heat, dehydration and the presence of toxic substances. In the present study, the slow rate of decline in viable rhizobia in the inoculated, but fungicide-free treatment indicates that the decline in rhizobial survival was due primarily to the toxicity of the various fungicides. The treated and inoculated seeds were kept at 4^{o}C, an optimum temperature for inoculant storage. At this

temperature dehydration also was minimal. The high recovery of viable *R. ciceri* from seeds treated with Crown could possibly be that more *Rhizobium* inoculant stuck to the seed coat since it was the only fungicide applied in liquid formulation. Another possible explanation for the slight effect of Crown on rhizobial viability could be that *R. ciceri* strain BCF 32 is tolerant to carbathiin and thiabendazole, the active ingredients. The rapid loss of viability with Apron (Revellin et al., 1993) and captan (Graham et al., 1980) treatments was reported previously for *B. japonicum* and *R. phaseoli*, respectively. Arrest (Thiram) showed a limited toxicity which supports the findings of others workers (e.g., Revellin et al., 1993).

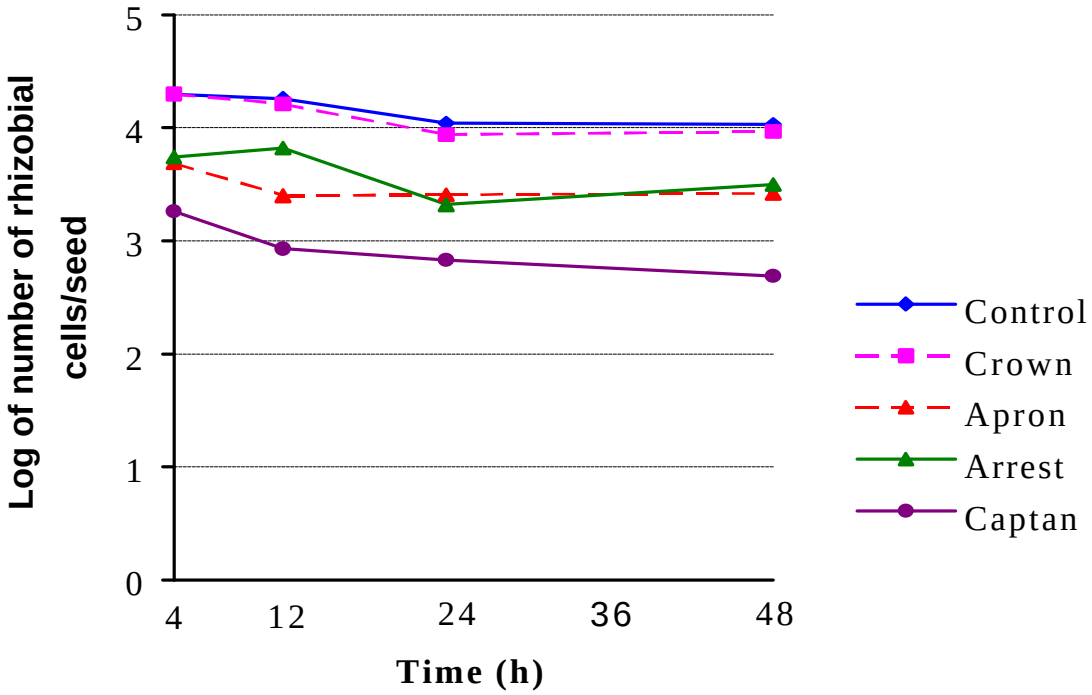

Fig. 1. Survival of *Rhizobium ciceri* strain BCF 32 on seeds treated separately with four fungicides 7 days prior to inoculation as compared to the inoculated, but fungicide-free control.

At the flowering stage, Arrest and the control treatment produced more nodules and a greater nodule dry weight than the other treatments but only crown reduced shoot dry matter yield (Table 2). However, at the early pod-filling stage, Apron had no effect on nodule dry weight. Captan treated seeds resulted in fewer nodules, a lower nodule dry weight and a lower shoot dry matter yield than for the fungicide-free control treatment. Crown and Arrest resulted in lower nodule dry weight and lower shoot dry weight than for the fungicide-free control treatment. The time of fungicide-*Rhizobium* contact had no effect on nodulation or shoot dry matter yield.

Table 2. Nodulation and dry matter production at the flowering and early pod-filling stages of chickpea plants grown from fungicide-treated seeds 7 days prior to inoculation and the inoculated, but fungicide-free, control.

	Flowering stage			Early pod-filling stage		
Treatment	No. of nodules (per 2 plants)	Nodule dry wt (mg/2 plants)	Shoot dry wt (g/2 plants)	No. of nodules (per 2 plants)	Nodule dry wt (mg/2 plants)	Shoot dry wt (g/2 plants)
Control	1.6	12.7	3.02	12.1	353.2	4.99
Crown	0.4	4.1	2.17	6.3	226.2	3.52
Apron	0.6	10.4	2.79	13.6	245.8	4.52
Arrest	1.3	22.1	2.86	7.3	219.6	3.92
Captan	0.6	5.1	2.57	4.6	243.9	4.02
LSD(05)	0.9	11.5	0.80	5.9	107.5	1.00

From the plate count tests, one can conclude that Crown is compatible with *R. ciceri* but nodulation and dry matter data suggest differently. Among the fungicides, Apron and Captan were most toxic to rhizobial survival, but they resulted in relatively high nodule dry weight and dry matter production at the early pod filling stage. The toxicity of these two fungicides may have persisted in the soil only for a short time period, after which the remaining viable cells rapidly multiplied and resulted in increased nodulation. Rhizobia lose their ability to induce nodulation before they lose their ability to multiply. This was demonstrated with Crown and to a lesser extent with Arrest, indicating that a viability test alone only provides a partial measure of compatibility.

In conclusion, fungicides affect inoculation to various degrees depending on the nature of the chemical involved. Of the fungicides tested, Apron had the least detrimental effect on inoculation by the early pod-filling stage. A method of inoculation which avoids direct fungicide-*Rhizobium* contact could be an alternative to seed inoculation. Hence, application of granular inoculant could be useful in kabuli chickpea production, in which case seed-treatment is required.

Acknowledgements
This work was supported by Saskatchewan Department of Agriculture and Food, Saskatchewan Pulse Growers and MicroBio RhizoGen.

References

Graham, P. H., Ocampo, G., Ruiz, L.D. and Duque, A. 1980 Survival of *Rhizobium phaseoli* in contact with chemical seed protectants. Agron. J. 72: 625-627.

Revellin, C., Leterme, P., Catroux, G. 1993 Effect of some fungicide seed treatments on the survival of *Bradyrhizobium japonicum* and the nodulation and yield of soybean (*Glycine max* L. Merr.). Boil. Fert. Soils 16: 211-214.

Vincent, J. M. 1970 A manual for the practical study of root nodule bacteria. Blackwell Scientific, Oxford, 164pp.