JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 44, No. 2 (2004)

INFLUENCE OF FUNGICIDE FUNABEN T ON NODULATION OF SOYBEAN [GLYCINE MAX (L.) MERR.] IN THE FIELD CONDITIONS

Krzysztof Pudełko, Cezary J. Mądrzak

August Cieszkowski Agricultural University, Department of Biochemistry and Biotechnology
Wołyńska 35, 60-637 Poznań, Poland
e-mail: kpudelko@owl.au.poznan.pl

Accepted: June 14, 2004

Abstract: Compatibility between rhizobial inoculants and fungicides used for seeds treatment remains controversial. The aim of this study was to investigate the effect of the fungicide Funaben T (thiram – 45%, carbendazim – 20%) on the nodulation of soybean (*Glycine max cv*. Nawiko) inoculated with two *Bradyrhizobium japonicum* strains (USDA 110 and USDA 123). Reduced nodulation of soybean was observed in the treatments with fungicide applied and fertilized with nitrogen (80 kg ha^{-1}). The strongest depression of nodulation was observed when both treatments were used simultaneously.

Key words: soybean, nodulation, fungicide, Funaben T, *Bradyrhizobium japonicum*

INTRODUCTION

Current practices used for legume production include inoculation of seeds with rhizobia to ensure effective nodulation and subsequent nitrogen fixation and treatment of the seed with fungicides to reduce seed rot and seedling damping-off resulting from infection by soil-borne pathogens. Although reports are conflicting, several studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium* (Welty et al. 1988; Ramos and Ribeiro 1993).

Revellin (1993) reported that Apron (metalaxyl) reduced viable *Bradyrhizobium japonicum* on soybean seed by 61%, while Captan and pentachloronitrobenzene reduced the viability of *B. japonicum* by 18% and 78%, respectively (Curley and Burton 1975). Less than 10% of *Rhizobium phaseoli* cells survived on seeds treated with Captan, after fungicide-rhizobia contact compared to more than 90% survival in a non-fungicide-treated control (Graham et al. 1980). The toxic effects of thiram on rhizobial survival have been reported (Graham et al. 1980; Hashem et al. 1997), but also contrarily no adverse effect on the survival of *B. japonicum* was found (Curley and Burton 1975). In the field studies, Captan adversely affected nodulation in inoculated chickpea (Welty et al. 1988), soybean (Tesfai and Mallik 1986), and pea (Rennie et al. 1985). Seed treatment with thiram reduced nodulation 40 days after planting in inoculated chickpea (Bhattacharyya and Sengupta 1984), but had no effect on nodulation and yield in other studies with chickpea (Thomas and Vyas 1984; Welty et al. 1988). A significant decrease in nodulation and yield of soybean was observed (Revellin et al. 1993) when the inoculated seeds were treated with Apron (metalaxyl), although the same chemical did not affect nodulation and yield in inoculated chickpea (Welty et al. 1988). Rennie et al. (1985) also reported reduced nodulation when either thiram or metalaxyl treated pea and faba bean seeds were inoculated. Furthermore, it was demonstrated that different species and strains of the same species of *Rhizobium* differed in their sensitivity toward various fungicides (Mallik and Tesfai 1983).

The objective of this study was to examine the effect of commercial fungicide Funaben T (45% thiram; 20% carbendazim) on nodulation effectiveness of two strains of *B. japonicum* applied on soybean seeds in the field conditions.

MATERIALS AND METHODS

The experiment was carried out in replicates on plots, 22.5 m^2 each and soybean (*Glycine max*. cv. Nawiko) was used as a host plant. The following treatments were tested: *B. japonicum* strains (USDA 110 and USDA 123) marked as A and B respectively; nitrogen fertilizer (80 kg ha^{-1}) marked as AN and BN (respectively to the bacterial strains used). All treatments were tested with and without fungicide. The soil was free of endogenous *B. japonicum* capable to nodulate soybeans. Seed inoculation was performed just before sowing by thoroughly mixing a peat based inoculum with embedded bacterial cultures and 3 mL of 1% (w/v) gum arabic solution (as a sticker) per 1 kg of seeds. Seeds were treated with the liquid solution containing 2 g of Funaben T per 1 kg of seeds one day before sowing.

RESULTS AND DISCUSSION

Number of nodules: From each plot 10 randomly selected plants were chosen; the number of nodules was counted and the results are presented in table 1.

It was observed that nitrogen fertilization (treatment AN and BN) as well as fungicide treatment significantly ($p < 0.05$) reduced the average number of nodules per plant. While the nodule number reduction due to the nitrogen application was not strain dependent (28 and 24.5% for treatment A and B, respectively), the influence of fungicide treatment on nodulation varied with the strain used for inoculation (31.5 and 45.7% reduction of nodulation for treatment A and B, respectively). Simultaneous use of nitrogen fertilization and fungicide significantly reduced the nodulation in both treatments, but the effect was stronger for strain USDA 123 (93.6% reduction of the nodules number) than for USDA 110 (52.4% reduction).

Nodules distribution: Since the location of the nodules on the plant root can give the certain indication of the infection process the distribution of nodules was analyzed with respect to tap root and lateral roots of the plant. The results are presented in table 1.

The proportion of the nodules located on the tap root was significantly reduced ($p < 0.1$) only on plants treated with fungicide where nitrogen fertilizer had not

Table 1. Effect of Funaben T treatment and nitrogen fertilization on number, size and distribution of nodule per soybean plant induced by *Bradyrhizobium japonicum* (*B. j.*) strains USDA 110 and 123

B. j. strain and nitrogen dose	Treatment designation	Average number of nodules per soybean plant		Proportion of nodules located on the tap root of the plant		Proportion of large nodules formed on plant roots	
		seeds treated with Funaben T	seeds untreated with Funaben T	seeds treated with Funaben T	seeds untreated with Funaben T	seeds treated with Funaben T	seeds untreated with Funaben T
B. j. USDA 110	A	7.15	4.9	75.96%	53.87%	100.00%	57.14%
B. j. USDA 123	B	5.15	3.4	63.05%	64.71%	75.53%	55.10%
B. j. USDA 110 + N 80 kg ha^{-1}	AN	4.7	2.55	77.24%	35.00%	59.22%	0.00%
B. j. USDA 123 + N 80 kg ha^{-1}	BN	3.55	0.3	89.35%	83.33%	33.80%	[a]66.67%

[a]not significant ($p > 0.1$) due to the low number of nodules

been used while on the treatments where nitrogen was applied the influence of the fungicide on the location of nodules was not statistically important ($p > 0.1$). These observations may suggest that in the conditions with the limited availability of nitrogen even the delayed nodulation (nodules located on the lateral roots) may be beneficial for the plants and the symbiotic relation can be established after rebuilding of the bacterial population size initially reduced by the fungicide application. These results correspond with the others suggesting that the major deleterious effects of the fungicides on rhizobial survival and plant growth occurred during the initial 4-h period of fungicide–*Rhizobium* contact (Kyei-Boahen 2001).

Size of nodules: Since it has been reported, that nodulation under severe conditions can lead to the reduction in nodule mass (Zahran 1999) the size of nodules formed on the plant root had been analyzed. The large and small nodules were counted separately and the proportion of large nodules out of total number of nodules formed is presented in table 1.

Both treatments (nitrogen and fungicide) caused the reduction of the nodules size formed on the plant root, however the extent of that effect differed with the bacterial strain used for inoculation. While for the treatments nodulated by USDA 110 strain the effect of nitrogen and fungicide applied separately for the nodule size was the same (AN without fungicide and A with fungicide respectively), the nodules size proportion in the treatments with USDA 123 strain was more influenced by nitrogen fertilization than fungicide use. Both treatments used simultaneously strongly reduced the nodule size and only the small nodules were formed on the plant root (AN with fungicide).

The observations presented herein suggest, that fungicide Funaben T used as seed treatment in the cultivation of soybean significantly depressed the nodulation effectiveness by reducing the nodule number and size as well as the distribution of the nodules on the plant root. The nitrogen fertilization effect (with the relatively high dose 80 $kgha^{-1}$) on the nodulation corresponds with the other authors reporting that nitrogen supplementation of the soil can reduce nodulation or nitrogen fixation effectiveness (Eardly et al. 1985, Streeter 1988; Abdel Wahab et al. 1996). Data presented here suggest also, that both treatments (nitrogen additive and fungicide) used simultaneously can depress the nodulation with higher extent than used separately. Although the application of Funaben T reduced the effectiveness of nodulation, introduced rhizobial strains were able to form the nodules on the plant roots. The possible risk of seed rot and seedling damping-off has to be considered together with the reduced benefits of efficient nitrogen fixation when the use of fungicide should be decided.

REFERENCES

Abdel Wahab A.M., Abd-Alla M.H. 1996. Effect of different rates of N-fertilizers on nodulation, nodule activities and growth of two field grown cvs. of soybean. Fert. Res., 43: 37–41.

Bhattacharyya P., Sengupta K. 1984. Effect of seed-dressing fungicides on nodulation and grain yield of chickpea. Int. Chickpea Newsl., 11: 41–44.

Curley R.L., Burton J.C. 1975. Compatibility of *Rhizobium japonicum* with chemical seed protectants. Agron. J., 67: 807–808.

Eardly B.D., Hannaway D.B., Bottomley P.J. 1985. Nitrogen nutrition and yield of seedling alfalfa as affected by ammonium nitrate fertilization. Agron. J., 77: 57–62.

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

Hashem F.M., Saleh S.A., van Berkum P., Voll M. 1997. Survival of *Bradyrhizobium* sp. (*Arachis*) on fungicide-treated peanut seed in relationship to plant growth and yield. World J. Microbiol. Biotechnol., 13: 335–340.

Kyei-Boahen S., Slinkard A.E., Walley F.L. 2001 Rhizobial survival and nodulation of chickpea as influenced by fungicide seed treatment. Can. J. Microbiol., 47: 585–589.

Mallik M.A.B., Tesfai K. 1983. Compatibility of *Rhizobium japonicum* with commercial pesticides *in vitro*. Bull. Environ. Contam. Toxicol., 31: 432–437.

Ramos M.L.G., Ribeiro W.Q., Jr. 1993. Effect of fungicides on survival of *Rhizobium* on seeds and the nodulation of bean (*Phaseolus vulgaris* L). Plant Soil 152: 145–150.

Rennie R.J., Howard R.J., Swanson T.A., Flores G.H.A. 1985. The effect of seed-applied pesticides on growth and N2 fixation in pea, lentil, and faba bean. Can. J. Plant Sci., 65: 23–28.

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

Streeter J. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. Crit. Rev. Plant Sci., 7: 1–23.

Tesfai K., Mallik M.A.B. 1986. Effect of fungicide application on soybean-rhizobia symbiosis and isolation of fungicide-resistant strains of *Rhizobium japonicum*. Bull. Environ. Contam. Toxicol., 36: 816–826.

Thomas M., Vyas S.C. 1984. Nodulation and yield of chickpea treated with fungicides at sowing. Int. Chickpea Newsl., 11: 37–38.

Welty L.E., Prestbye L.S., Hall J.A., Mathre D.E., Ditterline R.L. 1988. Effect of fungicide seed treatment and rhizobia inoculation on chickpea production. Appl. Agric. Res., 3: 17–20.

Zahran H.H 1999. *Rhizobium*-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. Microb. Mol. Biol. Rev., 63: 968–989.

POLISH SUMMARY

WPŁYW FUNGICYDU FUNABEN T NA BRODAWKOWANIE SOI [*GLYCINE MAX* (L.) MERR.] W DOŚWIADCZENIACH POLOWYCH

Oddziaływanie fungicydów stosowanych jako zaprawy nasienne na efektywność brodawkowania roślin przez rizobia, wprowadzone w formie inokulum, jest nadal przedmiotem kontrowersji. Celem niniejszej pracy było określenie wpływu fungicydu Funaben T (tiuram – 45%, karbendazym – 20%) na brodawkowanie soi (*Glycine max* cv. Nawiko) inokulowanej dwoma szczepami *Bradyrhizobium japonicum* (USDA 110 i USDA 123). Osłabione brodawkowanie zaobserwowano zarówno w doświadczeniach, w których zastosowano fungicyd jak i w tych, w których zastosowano nawożenie azotowe (w dawce 80 kg ha^{-1}). Najsilniejsze ograniczenie brodawkowania zaobserwowano w doświadczeniach, w których zastosowano obydwa czynniki jednocześnie.

Book Review

Praczyk T. 2003. Diagnostyka Uszkodzeń Herbicydowych Roślin Rolniczych [Diagnostic of Herbicide Damage to Cultivated Plants]. Państwowe Wydawnictwo Rolnicze i Leśne, Poznań, 144 pp. + 128 color photographs. ISBN 83-09-01804-5. (In Polish)

This book concerns very important problem in plant protection such as unintentional side effects of herbicide use in field crops. In part I "Introduction" (p. 5–6) the author indicates that the aim of the book is to provide advise and help to farmers in recognizing damage to cultivated plants unintentionally made by herbicides to their own crops or to the crops of their neighbors.

The book contains the following parts: II. – "Herbicide selectivity" (p. 7–8); III – "Herbicide classifications" (p. 9–12), IV – "Symptoms of plant damage due to herbicides" (p. 13–70), V – "Definitions" (p. 71–72), VI – "Literature" (p. 73), VII – "List of herbicides characterized in the book" (p. 74–78), VIII "Photographic documentation of symptoms on injured plants" (p. 79–144).

The most voluminous and useful are parts IV and VIII.

Part IV – "Symptoms of plant damage due to herbicides" (p. 13–70) contains characteristics of the following categories of herbicides: 1. Plant growth regulators (p. 14–23), 2. Inhibitors of amino acids synthesis (p. 23–34), 3. Inhibitors of lipid synthesis (p. 34–41), 4. Inhibitors of seedling growth (p. 41–51), 5. Inhibitors of photosynthesis (p. 52–64), 6. Inhibitors of pigment synthesis (p. 64–67), 7. Destructors of cell walls (p. 67–70).

Each chemical compound is well characterized and described with given IUPAC name, solubility characteristic, use range; lists of susceptible weeds and cultivated plants are included as well as the trade names of herbicides.

These descriptive data well correspond with color photographs included into part VIII which illustrate damage or injuries done by herbicides to cultivated plants.

The book will be of great help to farmers, extension workers and to those consultants who are asked by courts for opinion on plant damage causes and scale of financial compensation.

Jerzy J. Lipa
Institute of Plant Protection, Poznań, Poland