Effects of Crown (Carbathiin and Thiabendazole), Allegiance FL (Metalaxyl), Vitaflo 280 (Carbathiin and Thiram), and Apron Maxx (Fluodioxonil and Metalaxyl) on N_2 Fixation of Chickpea, Dry Bean, Lentil, and Pea.

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Introduction

Chickpea (*Cicer arietinum* L.), dry bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medikus), and pea (*Pisum sativum* L.) have the ability to fix dinitrogen (N_2) from the atmosphere. Rhizobium inoculants are applied to the seed to ensure effective N_2 fixation. In addition, fungicidal seed treatments are recommended to control extremely aggressive diseases such as ascochyta blight, anthracnose, seed rot, root rot, and seedling blight.

Information on the compatibility of Rhizobium inoculants with fungicidal seed treatments is controversial. Fungicide rate is the single most important factor causing variability of results. A survey of the most cited papers on the fungicide by Rhizobium interaction shows that only 14% used the recommended rate of Metalaxyl (5-g a.i. 100 kg^{-1} seed) (Table 1). Eighty six percent of papers report a rate of Metalaxyl ranging from 3.6- to 35-fold the recommended rate and only one found a decrease in soybean nodulation caused by Metalaxyl when used at 35-fold the recommended rate (Revellin et al., 1993).

A Similar survey on Thiram shows that only 27% of the reports used the recommended rate (50-g a.i. 100 kg^{-1} of seed) and found no effect of Thiram on nodulation of chickpea and soybean (Curley and Burton, 1975; Bhattacharyya and Sengupta, 1980) or nodulation and nitrogen fixation of soybean (Rennie and Dubetz, 1984). Seventy three percent of papers report a rate of Thiram ranging from 2- to 16-fold and only two found a decrease in nodulation of chickpea and soybean caused by Thiram when used at 4.6-fold and 2-fold the recommended rate, respectively (Bhattacharyya and Sengupta, 1980; Rennie and Dubetz, 1984). In addition, Thiram reduced N_2 of chickpea when applied at 2.8-fold the recommended rate (Kyei-Boahen et al., 2001).

Contradictory results have been reported among laboratory, growth chamber, and field evaluations of Rhizobium strains in contact with fungicides (Curley and Burton, 1975; Hashem et al., 1997; Dunfield et al., 2000; Kyei-Boahen et al. 2001). Therefore, laboratory tests provide a measure of Rhizobium survival that sometimes does not correlate with growth chamber or field results. The objective of this study was to determine the effects of Crown, Allegiance FL, Vitaflo 280, and Apron Maxx at recommended rates on visual nodulation and percent N_2 derived from the atmosphere (Ndfa) of chickpea, dry bean, lentil, and pea.

Materials and Methods

Chickpea cv. Sanford, dry bean cv. CDC Camino, lentil cv. Laird, and pea cv. CDC Mozart were grown at Clavet and Langham, Saskatchewan 2002. At these locations, pulse crops were never grown before. Soil type, soil available nitrogen, total precipitation, seeding date, and harvest date are given in Table 2. Chickpea was inoculated with *Rhizobium ciceri* [TagTeam (Philom Bios) and SelfStick chickpea (Becker Underwood)]. Dry bean was inoculated with *Rhizobium leguminosarum* bv. *phaseoli* [TagTeam (Philom Bios) and SelfStick dry bean (Becker Underwood)]. Lentil and pea were inoculated with *Rhizobium leguminosarum* bv. *viceae* [N-Prove (Philom Bios) and SelfStick lentil and pea (Becker Underwood)]. Rhizobium inoculants were applied at the manufacturer recommended rate. The active ingredients and fungicide rates are given in Table 3.

Treatments for chickpea were: 1) Rhizobium inoculated, no fungicide, 2) Allegiance FL, Rhizobium applied sequentially to fungicide, 3) Crown plus Allegiance FL, Rhizobium applied sequentially to fungicides, 4) Crown plus Allegiance FL, Rhizobium applied simultaneously to fungicides, and 5) Allegiance FL, no Rhizobium inoculant. Treatments for dry bean were: 1) Rhizobium inoculated, no fungicide, 2) Vitaflo 280 plus Allegiance FL, Rhizobium applied sequentially to fungicides. 3) Apron Maxx, Rhizobium applied sequentially to fungicide and, 4) No Rhizobium, no fungicide. Treatments for lentil were: 1) Rhizobium inoculated, no fungicide, 2) Vitaflo 280, Rhizobium applied sequentially to fungicide, 3) Crown, Rhizobium applied sequentially to fungicide, 4) Vitaflo 280, Rhizobium applied simultaneously to fungicide, 5) Crown, Rhizobium applied simultaneously to fungicide, and 6) No Rhizobium, no fungicide. Treatments for pea were: 1) Rhizobium inoculated, no fungicide, 2) Vitaflo 280 plus Allegiance, Rhizobium applied sequentially to fungicides, 3) Vitaflo 280 plus Allegiance FL, Rhizobium applied simultaneously to fungicides, 4) Apron Maxx, Rhizobium applied sequentially to fungicide, and 5) No Rhizobium, no fungicide.

The percent Ndfa was determined by the ^{15}N natural abundance method (Bremer and van Kessel, 1990,) using flax as a reference crop. Plant samples were harvested at mid-podding stage, dried to constant weight, and finely ground to talcum powder consistency. Isotopic composition ($d^{15}N$) was determined in an automated single-inlet system (RoboPrep Sample Converter interface with a TracerMass Stable Isotope detector, Europe Scientific, Crewe, England). Visual nodulation was measured at mid-podding stage using a scale 0-4. A zero was assigned to plants with no nodulation and a four to plants with good nodulation. Visual nodulation was assessed by a combination of nodules number, size, and colour. Data were analysed using the General Lineal Model (SAS Institute Inc. 1996). Seeding was delayed at both locations due to dry soil conditions and low average precipitation during May and early June (Table 2). The combination of late maturity and grasshoppers damage did not allowed biomass or grain yield data collection. The location by treatment interaction was not significant for any of these crops. Therefore, the means from the combined analysis of variance for visual nodulation and percent Ndfa are presented.

Results and Discussion

Fungicidal seed treatments had no effect on visual nodulation and percent Ndfa of chickpea, dry bean, lentil, and pea (Table 4). Visual nodulation and percent Ndfa of chickpea, dry bean, lentil, and pea treated with fungicides and inoculated with Rhizobium was similar to that observed in these crops inoculated with Rhizobium and non-treated with fungicides. Rhizobium inoculation of chickpea, dry bean, lentil, and pea significantly increased visual nodulation and percent Ndfa when compared with the non inoculated control, suggesting that these soils may not have a native Rhizobium strain capable of efficiently nodulate these crops (Bremer et al., 1988).

Our results agree with other studies conducted under controlled environments and under field conditions. Thus, it has been reported that Metalaxyl has no effect on nodulation of chickpea (Welty et al., 1988; Kyei-Boahen et al., 2001), lentil (Rennie et al., 1985), and pea (Rennie et al., 1985; Kutcher et al. 2002). Other reports indicate that Metalaxyl has no effect on the ability of Rhizobium to fix nitrogen from the atmosphere on alfalfa (Edmisten et al., 1988), chickpea (Kyei-Boahen et al., 2001), lentil (Rennie et al., 1985), and pea (Rennie et al., 1985). Similarly, Thiram had no effect on nodulation of chickpea (Welty et al., 1988), bean (Graham et al., 1980), lentil (Rennie et al., 1985), pea (Rennie et al., 1985; Fritz and Rosen, 1981; Dunfield et al., 2000; Kutcher et al., 2002), and soybean (Rennie and Dubetz, 1984). These reports support the finding of this study, that Metalaxyl and Thiram at the recommended rates are compatible with Rhizobium inoculants.

Others have shown that fungicides reduced nodulation or N2 fixation (Bhattacharyya and Sengupta, 1980; Revellin et al., 1993; Kyei-Boahen et al., 2001). However, in those studies they applied a rate of fungicide that exceeded the recommended rate by two- to 35-fold and consequently their results must be viewed with caution (Table 1). For example, Revellin et al. (1993) reported a negative effect of Metalaxyl on nodulation of bean. However, the Metalaxyl rate used by Revellin et al. (1993) was 35 fold higher than the recommended rate. Nodulation of chickpea was not affected by Thiram at recommended rate, but decreased significantly when applied at 4.6 fold the recommended rate (Bhattacharyya and Sengupta, 1980). Similarly, Thiram had no effect on soybean nodulation when applied at the recommended rate but decreased nodulation when applied at two fold the recommended rate (Rennie and Dubetz, 1984).

Conclusion

Allegiance FL, Crown, Vitaflo 280, and Apron Maxx at the recommended rates have no effect on visual nodulation or the ability of the Rhizobium to fix N_2 from the atmosphere, regardless of the inoculation time.

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Table 1. Effect of Metalaxyl, Thiram, and Crown on nodulation and nitrogen fixation of alfalfa, chickpea, dry beans, lentil, pea, and soybean.

Authors	Crop	Metalaxyl Rate used	Thiram Rate used	Crown Rate used	Effect on Nodulation	Effect on N_2 fixation
Edmisten et al., 1988. Crop Sci. 28: 568-570	Alfalfa	Recommended*	--	--	--	No effect
Welty et al, 1988. Applied Agron. Res. 3(1) :17-20	Chickpea	3.6-fold higher	12.3-fold higher	--	No effect No effect	--
Bhattacharyya and Sengupta, 1980. Int. Chickpea Newsl. 11:41-44	Chickpea	--	4.6-fold higher Recommended	--	Decreased No effect	--
Kyei-Boahen et al., 2001. Can. J. Microbiol. 47:585-589	Chickpea	14-fold higher	-- 2.8-fold	-- 6 fold higher	No effect No effect No effect	No effect Decreased Decreased
Graham et al., 1980. Agron. J. 72:625-627.	Beans	--	2-fold higher	--	No effect	--
Rennie et al., 1985. Can. J. Plant Sci. 65:23-28	Lentil	4-fold higher	-- 2-fold higher	--	No effect No effect	No effect No effect
Rennie et al., 1985. Can. J. Plant Sci. 65:23-28	Pea	4-fold higher	-- 2-fold higher	--	No effect No effect	No effect No effect
Fritz and Rosen, 1991. Can. J. Plant Sci. 71:1271-1274.	Pea	--	16-fold higher	--	No effect	--
Dunfield et al., 2000. Biol. Fertil. Soils 31:303-309	Pea	--	0.5- to 4-fold higher	--	No effect	No effect
Kutcher et al., 2002. Can. J. Plant Sci. 82:645-651	Pea	9.3-fold higher	-- 2-fold higher	--	No effect No effect	--
Curley and Burton, 1975. Agron. J. 67:807-808.	Soybean	--	Recommended	--	No effect	--
Rennie and Dubetz, 1984. Agron. J. 76:454	Soybean	--	2-fold Recommended	--	Decreased No effect	No effect No effect
Revellin et al., 1993. Biol. and Fert. Soils 16:211-214	Soybean	35-fold higher	-- --	-- --	Decreased --	-- --

*, Recommended rates: Metalaxyl 5-g a.i, Thiram 50-g a.i., and Crown at 300-mL per 100 kg of seed, respectively.

Table 2. Soil type, soil available nitrogen (0-12 cm), total precipitation from May to August, and seeding date of pulse crops grown at two locations in Saskatchewan in 2002.

Locations	Soil type	$N0_3$-N (Kg ha^{-1}) (0-12 cm)	Precipitation May to August (mm)	Seeding date	Harvest date
Clavet	Clay loam	30	150	June 14, 2002	No harvest
Langham	Sandy loam	21	180	June 13, 2002	No harvest

Table 3. Active ingredients and rates of fungicidal seed treatments used in chickpea, dry bean, lentil, and pea grown at two locations in Saskatchewan in 2002.

Crops	Crown	Allegiance FL	Vitaflo 280	Apron Maxx
Active ingredient	Carbathiin (Systemic activity) Thiabendazole (Contact and systemic activity)	Metalaxyl (Systemic activity)	Carbathiin (Systemic activity) Thiram (Contact activity)	Fluodioxonil (Contact activity) Metalaxyl (Systemic activity)
Chickpea	300-mL/100 kg of seed	16-mL/100 kg of seed		
Dry Bean		16-mL/100 kg of seed	260-mL/100 kg of seed	325-mL/100 kg of seed
Lentil	600-mL/100 kg of seed		330-mL/100 kg of seed	
Pea		16-mL/100 kg of seed	260-mL/100 kg of seed	325-mL/100 kg of seed

Table 4. Treatments means for visual nodulation and percent nitrogen derived from the atmosphere for chickpea, dry bean, lentil, and pea grown in Saskatchewan at two locations in 2002.

Crop	Treatment	Visual nodulation Scale (0-4)	Nitrogen derived from the atmosphere (%)
Chickpea	1. Rhizobium, no fungicide	2.31a	36a
	2. Allegiance FL, inoculated sequentially	2.19a	37a
	3. Crown plus Allegiance FL, inoculated sequentially	2.44a	31a
	4. Crown plus Allegiance FL, inoculated simultaneously	2.19a	31a
	5. No Rhizobium plus Allegiance FL	0.31b	11b
	LSD (0.05)	**0.64**	**8**
Dry Bean	1. Rhizobium, no fungicide	2.65a	42a
	2. Vitaflo 280 plus Allegiance FL, inoculated sequentially	2.47a	46a
	3. Apron Maxx, inoculated sequentially	2.84a	48a
	4. No Rhizobium, no fungicide	0.16b	14b
	LSD (0.05)	**0.93**	**24**
Lentil	1. Rhizobium, no fungicide	3.06a	45a
	2. Vitaflo 280, inoculated sequentially	3.00a	40a
	3. Crown, inoculated sequentially	2.67a	46a
	4. Vitaflo 280, inoculated simultaneously	2.57a	45a
	5. Crown, inoculated simultaneously	2.67a	41a
	6. No Rhizobium, no fungicide	0.31b	22b
	LSD (0.05)	**0.50**	**12**
Pea	1. Rhizobium, no fungicide	3.06a	54a
	2. Vitaflo 280 plus Allegiance FL, inoculated sequentially	3.25a	45a
	3. Vitaflo 280 plus Allegiance FL, inoculated simultaneously	2.81a	44a
	4. Apron Maxx, inoculated sequentially	2.75a	47a
	5. No Rhizobium, no fungicide	0.36b	27b
	LSD (0.05)	**1.0**	**11**