BENTAZON-MCPA EFFECT ON *Fusarium oxysporum* ROOT ROT ON *Trifolium pratense* IN GREENHOUSE CONDITIONS

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ABSTRACT

In Chile, the main factor in red clover (*Trifolium pretense* L.) stand decline is fusarium-root rot, which results in a reduction in yield. *Fusarium oxysporum* (Schlect.) is the most prevalent pathogen recovered from diseased red clover roots plants. Agronomical management of red clover includes application of broadleaf herbicides such as MCPA and bentazon. This study was conducted to evaluate the effects of bentazon and bentazon/MCPA mixture on phytotoxicity and root rot as caused by *F. oxysporum* on red clover, under greenhouse conditions. In addition, in vitro mycelial growth and conidial germination of *F. oxysporum* was studied. A reduction of 40% in crown diameter and 57% shoot dry weight was observed 30 days after treatment with a high rate of bentazon/MCPA and *F. oxysporum* inoculum. The bentazon - *F. oxysporum* interaction reduced root dry weight by 57% at 1X rate and 42% with 2X rate at 20 and 30 of evaluation. Bentazon caused a significant increase in phytotoxicity at the high rate and the mixture bentazon/MCPA increased root rot severity and phytotoxicity at the high rate. The conidial germination and mycelial growth were significantly reduced by bentazon/MCPA. These results suggest that red clover growth and persistence could be adversely affected by *F. oxysporum* after bentazon and bentazon/MCPA application.

Keywords: herbicide, fusariosis, legume, plant diseases, phytotoxicity

INTRODUCTION

Herbicides are reported to affect the incidence and severity of plant diseases trough their interaction with plants, pathogens or other microorganisms (Heydari *et al*., 1997; Heydari and Misaghi, 2003). While a number of plant diseases are reported to occur with increased incidence and severity, after the application of herbicides (Altman and Campbell, 1977; Altman and Rovira, 1989; Bollen, 1993; Sanogo *et al*., 2000), other diseases have shown decreases or no significant changes (Dann *et al*., 1999; Dissanayake *et al*., 1998).

Fusarium root rot is a common disease in Chilean red clover pastures, as well as in other areas in the world (Ceballos *et al*., 2004, 2006; Galdames, 1991; Steiner and Alderman, 1999; Venuto *et al*., 1995), causing reductions in productivity and persistence (Coulman and Lambert, 1995; Leath, 1985; Venuto *et al*., 1996). *Fusarium oxysporum* Schldt. is the most common, economically important and studied Fusarium species (Ceballos *et al*., 2004; 2006; Venuto *et al*., 1995; Venuto *et al*., 1999). Fusarium affected plants may exhibit poor emergence, and the resulting seedlings are often stunted and weak. The pathogen penetrates directly through wounds in the plant surface, causing necrotic lesions on lower stems and roots (Carson *et al*., 1991).Akiyama *et al*., 2002; Larose *et al*., 2002; Vierheilig *et al*., 2002).

Commercial formulation of MCPA [(4-chloro2-methylphenoxy) acetic acid)] and bentazon (3-isopropyl-1H-2,1,3-benzo-thiadiazin-4(3H)-one 2,2-dioxide), are widely used in Chile, and worldwide (AFIPA, 2002; Kuds and Streibig, 2003; Liu *et al*., 1994) to control broadleaf weeds in red clover. MCPA is absorbed by roots and foliage and translocated into the plant and causes the death of susceptible weeds. It stimulates uncontrolled growth of the meristematic tissues and diminish synthesis of DNA and proteins; thus disrupting basic metabolic processes in plant cells and tissues. While these effects are lethal to susceptible weeds, they may also affect tolerant crops depending on a number of factors (Conrad and Stritzke, 1980). Research on cereal crops has indicated that low concentrations of chlorinated phenoxyacetic acid derivatives may stimulate the rate of root and shoot growth, seed germination and photosynthesis, but the higher concentrations inhibit these processes (Grabinska-Sota *et al.*, 2003; Hess, 1993). MCPA causes stunting, distortion and gall formation on the roots of a number of crops (Tharp and Kells, 2000). Studies on MCPA used for weed control on red clover in Chile indicated that this herbicide had the most detrimental effects on red clover plants (Ceballos *et al.*, 2004). On the other hand, bentazon is a contact herbicide that inhibits photosynthesis by specifically inhibiting photosystem II (Bradshaw *et al*., 1992; Fleming *et al*., 1988). The primary mechanism of bentazon action is to compete for the QB binding site with plastoquinone, the electron transporter in photosystem II, resulting in photosynthesis inhibition and generation of oxidative stress in sensitive species (Wu and Wang, 2003). Bentazon has very little long distance systemic activity within the plant and acts directly where is applied and is absorbed primarily by the foliage but also by the roots (Tomlin, 1995). This herbicide is used only as a post-emergency herbicide, for control of annual and perennial broadleaf weeds (AFIPA, 2002).

The phytotoxic effect of MCPA, bentazon, 2,4-DB, flumetsulam, haloxifop-methyl, and of the mixtures usually used on red clover as flumetsulam/2,4-DB and bentazon/MCPA herbicides have been reported (Ceballos et al., 2004). In our study, all the herbicides were evaluated at recommended and twice recommended rate and all caused early foliar damage, but bentazon and bentazon/MCPA produced the greatest shoot damage. Roots were injured from all herbicides, yet usually recovered over time. Bentazon/MCPA induced the most severe root injury, up to 17% of the plants, and caused seedling death (Ceballos *et al*.,

2004). In a subsequent study, the influence of MCPA on *F. oxysporum* root rot on red clover showed increased severity of Fusarium root rot on red clover seedlings and phytotoxicity at a high rate (Ceballos *et al.*, 2006). However, there is no information available on the effects of bentazon and a bentazon/MCPA mixture on *F. oxysporum* and their role in the severity of red clover root rot caused by *F. oxysporum*. Therefore, the objective of this study was to evaluate the effects of bentazon and bentazon/MCPA on Fusarium root rot and red clover growth under greenhouse conditions.

MATERIAL AND METHODS

Greenhouse studies of the herbicide effects on Fusarium-disease

This study was carried out in two independent experiments. Each experiment consisted of three rates of bentazon (0, 750 and 1500 g a.i. ha^{-1}) and bentazon/MCPA (0, 750/720, 1500/1440 g a.i. ha-1) corresponding to 0 (control), field rate (1X) and twice of field rate (2X). The herbicide treatments were applied, with a compressed-air bicycle sprayer calibrated to deliver 200 L ha^{-1} of sterile water solution, to seedlings at the two- to three-leaf trifoliate stage. (Ceballos *et al*., 2006).

For all experiments the plants were grown as describe by Ceballos et al. (2004; 2006) and Islam and Weil (1998). Two seeds, previously disinfected with a 0.5% sodium hypochlorite solution, were placed in each pot with allophanic soil (pH 6.1, 17% organic matter, 31.6% sand, 53% silt, 15.4% clay) and were fertilized during the experiment (N 50; P 200, K 80 mg kg^{-1}).

F. oxysporum isolates were recovered and produced from diseased red clover plants, with evident vascular wilt symptoms, at the Carillanca Experimental Station in Temuco, Chile. Symptomatic root rot tissue was cut to 1 cm length, washed in distilled water, and soaked in a solution of 1% NaOCl for 3 min (Akinsanmi *et al*., 2004). A 1.0 cm subsection was cut from each sterilized section and placed in Petri plates containing potato-dextrose agar (39 g L^{-1} of Difco PDA) and streptomycin sulphate (100 mg L^{-1}). Plates were incubated at 25ºC for 14 d. The pathogenicity of isolates was assays on the Quiñequeli red clover cultivar, and the most pathogenic isolate, F127, was transferred to fresh PDA to complete the Koch's postulates and to continue the experiments (Ceballos *et al.*, 2006). The specific characterization of this strain as *F. oxysporum* (IMI 390980) was provided by CABI Bioscience Identification Services, UK Centre (Egham). Inoculum was produced according to methods employed by Sanogo *et al*. (2000) and Ceballos et al. (2006). An agar plug, 5 mm diameter of selected isolate, was transferred from the colony of each isolate to PDA-plates and incubated at 22°C. After 14 d, conidia were harvested by adding 15 mL of sterile distilled water to each plate and gently scraping the medium surface with a paint brush. The number of conidia was determined with a hemacytometer.

The inoculum consisted of two levels, 0 (untreated plants) and level 1 (5 mL of $2.5x10^6$ spores m L^{-1}) and was applied, 15 d after herbicide treatments, by injection into the root zone of the aseptically grown plants at the two to three-leaf trifoliate stage. Temperature was 20-25ºC between night and day respectively and light was supplemented with 1000 w lamps for 16 h.

Pots were watered regularly on the surface throughout the experiment to ensure adequate soil moisture. Seedlings were carefully harvested at 20 and 30 d after herbicide treatment and washed under running tap water. Root length, seedling height, crown diameter and dry weight of shoots and roots were measured.

The effects of herbicides on fusarium pathogenicity and plant phytotoxicity were assessed on a scales proposes by Ceballos *et al*. (2006). Fusarium pathogenicity the scale was from 1 to 4, where 1 = completely healthy root tissue; 2 = a few superficial dark-brown lesions of root tissue; 3 = extended superficial dark-brown lesions of root tissue and 4 = necrotic root or tissue death. For herbicide phytotoxicity on red clover plants scale was from 1 = no visible damage to 5 = complete plant death, with symptoms that included chlorosis, leaf necrosis, and stunted growth (Ceballos *et al*., 2004, Ceballos *et al*., 2006). The root disease severity index (RDSI) and foliage damage index (FDI), were calculated by according to Vettraino *et al*. (2003) and Ceballos *et al*. (2006).

Effects of herbicides on *in vitro* mycelial growth

This study was carried out at optimal nutritional conditions for mycelial growth of *F. oxysporum* (Sanogo *et al*., 2000). Potato-dextrose agar was amended with the commercial formulation of bentazon and bentazon/MCPA at 0, 1X and 2X rates. An agar plug, 5 mm diameter, was removed from the margin of the actively growing colony of F127 (2-wk-old cultures) placed in the center of herbicide-amended plates, and incubated at 22°C with a 12 h photoperiod supplemented with a fluorescent light (Ceballos *et al*., 2006). Radial growth of mycelium from the center was recorded regularly over a month. The experiment was arranged in a completely randomized design with six replications for treatment.

Effects of herbicides on *in vitro* conidial germination

For conidial germination studies, conidia were obtained and collected by PDA cultures prepared as described in the fungal isolation section. Collected conidia were suspended in a solution of each herbicide treatment and incubated at 22°C in darkness. A treatment with conidia suspended in distilled water, at the same incubation conditions, was incorporated as control. After 6 h the number and proportion of germinated of conidia was determined under microscope with a hemacytometer (Sanogo *et al*., 2000). The proportion of germinated conidia was computed as the ratio of germinated conidia to observed conidia. The experiment was arranged in a completely randomized design with six replications for treatment.

Data analysis

The greenhouse trials were analyzed as completed randomized designs using a standard analysis of variance procedure. Treatments were considered fixed effects and reps within treatment were random and used for the error term. Means for root length, seedling height, crown diameter and dry weight of shoots and roots were separated using a Tukey's multiple range test at a 5% significance level. Since foliage damage and root rot severity were evaluated using rating scales, resulting in ordinal data. These were analyzed using the Kruskal-Wallis

test, one-way analysis based on ranks, and group ranks were separated using the Conover-Inman test ($P \leq 0.05$) (Conover, 1999; Ceballos *et al.*, 2004; Shah and Madden, 2004). Data of mycelial growth are given as a growth index obtained by dividing average diameter growth for the treatment by that for the corresponding control. Results were analyzed as a completely randomized design with treatments considered fixed effects and reps within treatment were random and used as the error term. Growth index means were separated by Dunnet's multiple comparisons test with a significance level of $P \leq 0.05$. Conidial germination means were separated using Tukey's test ($P \leq 0.05$).

RESULTS

Although herbicides are applied to protect plants, they may also affect soil properties, microorganism, and host. These effects normally have little influence on plant growth. However, occasionally they cause temporary reduction in plat growth. This topic is complicated by the complex interactions of herbicide dose, formulation, tillage systems, environmental conditions, the plant pathogen, and the plant (Altman and Campbell, 1977; Altman and Rovira, 1989; Bollen, 1993). Furthermore, the timing of infection with the pathogen vs. that of the herbicide treatment can have a profound influence on the interaction. Thus, the literature often appears to be conflicting, but the apparent conflicts may be due to differences in one or more of the factors involved (Altman and Campbell, 1977; Bollen, 1993). Indirectly, through their strong effects on plants, herbicides can influence almost any process or interaction of the plant, including its susceptibility to plant diseases. In some cases, herbicides also have direct effects on plant pathogens (Bollen, 1993; Heydari *et al.*, 1997; Heydari and Misaghi, 2003; Ceballos *et al*, 2006).

F. oxysporum interaction with herbicide treatments

Analysis of variance indicated that the bentazon - *F. oxysporum* interaction was statistically significant for root dry weight, and that the effects of these factors were not independent during the experimental period. For plant height, root length and shoot dry weight this interaction was not significant, but separately herbicide rate and inoculum level has a significant effect on these plant-growth parameters (Table 1). Crown diameter was not affected by herbicide rate, inoculum level or the interaction among these factors.

The interaction of bentazon rate and *F. oxysporum* inoculums level for root dry weight was statistically significant, indicating that bentazon rates and inoculums levels were not independent during the experiment. Across the 20 and 30 days evaluations, the mean reduction in root dry weight due to *F. oxysporum* alone was 37%, compared to control, and the combination of reduced root dry weight by 42 and 49% at the 1X and 2X rates, respectively, in comparison with the mean of the untreated plants (Table 2). However, the reduction due to bentazon and *F. oxysporum* was different from bentazon alone for the 1X rate at 20 days only (Tukey's test, $P \leq 0.05$) (Table 2).

Plants treated with bentazon had 17% and 19% reduction in plant height at 20 days for 1X and 2X rate respectively, compared to the untreated plants (Table 1).

Table 1: Bentazon effect on plant height, root length and shoot dry weight of red clover plants.

Rates [a]	Plant height (cm)	Root length (cm)	Shoot dry weight (mg)
20 d			
Control	20,8a[b]	20,7b	247,1a
1X	17,3ab	21,6b	174,1ab
2X	16,8b	26,4a	151,1b
30 d			
Control	19,7a	18,1a	233,3a
1X	19,5a	21,0a	170,6a
2X	18,6a	17,1a	202,1a

[a] Rate 1X: recommended; rate 2X: double recommended rate. [b] Values within a column, for each time period, followed by the same letter are not significantly different based on Tukey's test ($P \leq 0.05$).

Table 2: Bentazon rate and *Fusarium oxysporum* interaction effects on red clover root dry weight (mg) at 20 and 30 days after herbicide treatment

Inoculum level [a]	Bentazon rate [b]		
	Control	1X	2X
20 d			
Untreated	95,1a[c]	52,5c	48,6c
Inoculated	65,3b	60,0b	40,8c
30 d			
Untreated	103,1a	55,5b	50,5b
Inoculated	59,5b	53,6b	59,9b

[a] Inoculums level: Untreated: 5 mL of distilled water; Inoculated: 5 mL of $2.5x10^6$ spores mL^{-1} conidial suspension of *F. oxysporum*. [b] Rate 1X: recommended rate; rate 2X: twice of recommended rate. [c] Values followed by the same letter are not significantly different based on Tukey's test ($P \leq 0.05$).

However, this difference was significant for the 2X rate only. Root length at 20 days was 4% and 28% increased by 1X and 2X rate, respectively, but only the 2X rate was statistically different from the control plants (Table 1). Shoot dry weight at 20 d was 30% and 39% reduced by 1X and 2X rates, respectively, however again no significant difference was observed between the control and the 1X rate of bentazon. At 30 days there were no differences observed for plant height, root length and shoot dry weight between the control and either rate of bentazon. There was not significant bentazon/MCPA- *F. oxysporum* interactions for plant height and root length, demonstrating that the effects of both factors were independent

throughout the experiment. Crown diameter was reduced by application of bentazon/MCPA 2X rate by 30% and 19% at 20 and 30 d, respectively (Table 3), but this reduction was statistically significant only at 20 d. Plants treated with 2X rate of bentazon/MCPA also had 45% and 41% reduction in plant shoot dry weight at 20 and 30 d, respectively, compared with untreated plants (Table 3). *F. oxysporum* inoculation significantly affected crown diameter, shoot dry weight and root dry weight. Crown diameter of red clover was reduced 17 and 5% at 20 and 30 d respectively, compared with untreated plants (Table 4). However, this reduction was statistically significant at 20 days only (P $P \leq 0.02$). Shoot dry weight of plants inoculated with *F. oxysporum* was reduced 37 and 29% at 20 and 30 d respectively and plant root dry weight was reduced 41 and 35% at 20 and 30 d, respectively, compared to the untreated control (Table 4).

Table 3: Bentazon/MCPA effect on crown diameter, shoot and root dry weight of red clover plants.

Rates [a]	Crown diameter (mm)	Shoot dry weight (mg)	Root dry weight (mg)
20 d			
Control	2,7a [b]	247,1a	80,2a
1X	1,7b	132,1b	37,2b
2X	1,9b	136,7b	40,0b
30 d			
Control	2,1a	233,4a	76,7a
1X	1,7a	202,8a	48,0b
2X	1,7a	138,2b	37,5c

[a] Rate 1X: recommended rate; rate 2X: twice of recommended rate. [b] Values followed by the same letter are not significantly different based on Tukey's test ($P \leq 0.05$).

Table 4: *Fusarium oxysporum* effect on crown diameter, shoot and root dry weight of red clover plants.

Inoculums level [a]	Crown diameter (mm)	Shoot dry weight (mg)	Root dry weight (mg)
20 d			
Untreated	2,3a	210,2a	66,0a
Inoculated	1,9b	133,3b	38,9b
30 d			
Untreated	1,9a	223,4a	68,0a
Inoculated	1,8a	159,4b	44,3b

[a] Inoculums level: Untreated: 5 mL of distilled water; Inoculated: 5 mL of 2.5×10^6 spores mL^{-1} conidial suspension of *F. oxysporum*. Values followed by the same letter, for each parameter and time, are not significantly different based on Tukey's test ($P \leq 0.05$).

At 30 d the interactions of bentazon/MCPA-Fusarium to crown diameter and shoot dry weight were significant, indicating that bentazon/MCPA rates and inoculum levels were not independent of each other in this experimental period. Compared with untreated plants, crown diameter was reduced 43 and 40% at the 1X and 2X rate, respectively, by bentazon/MCPA in presence of F. oxysporum inoculation (Table 5).

Fusarium-inoculated plants had 39% and 57% reduction in plant shoot dry weight with 1X and 2X of bentazon/MCPA rates in the presence of F. oxysporum inoculum compared to untreated control (Table 5).

Table 5. Bentazon/MCPA rate and *Fusarium oxysporum* inoculation interaction effects on red clover crown diameter and shoot dry weight at 30 days following herbicide treatment.

Inoculums level [a]	**bentazon/MCPA rate** [b]		
Crown diameter (mm)	Control	1X	2X
Untreated	2,8a[c]	1,8b	1,8b
Inoculated	1,8b	1,6c	1,7b
Shoot dry weight (mg)			
Untreated	279,2a	235,7ab	155,6c
Inoculated	267,6a	169,8b	120,5c

[a] Inoculums level: Untreated: 5 mL of distilled water; Inoculated: 5 mL of $2.5x10^6$ spores mL^{-1} conidial suspension of *F. oxysporum*. [b] Rate 1X: recommended rate; rate 2X: twice of recommended rate.

[c] Values followed by the same letter are not significantly different based on Tukey's test ($P \leq 0.05$).

Foliage damage and root rot indices

The foliage damage index (FDI) displayed statistically significant differences for inoculated plants; this response was not independent of the herbicide application rate or days after treatment (Figure 1B). At 20 d, bentazon significantly increased FDI at the 2X rate, but at 30 d, differences at the 2X rate were not statistically significant (Figure 1A). Detrimental effects were observed on foliage of red clover plants with application of bentazon/MCPA throughout the experimental period for all rates (Figure 1B). At 20 d the F. oxysporum plus 1X bentazon/MCPA rate caused the most phytotoxic effect and the FDI was 120% higher relative to untreated plants (Figure 1B).

At 30 days after bentazon application the root rot severity did not vary significantly among treatments (Figure 1C). However, the inoculated plants 20 d after bentazon application demonstrated an increased root disease severity index (RDSI). However not significant differences were observed between rates (Figure 1). The RDSI of plants treated with the herbicide mixture bentazon /MCPA were significantly affected throughout the experimental period (Figure 1D). The inoculated plants treated with 1X and 2X rates of

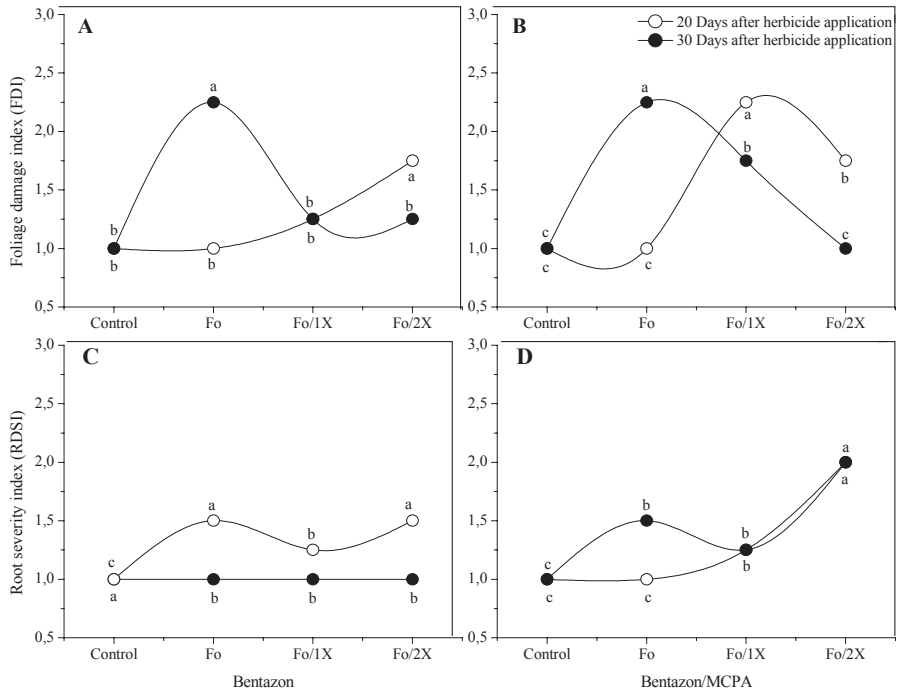

Figure 1: Foliage damage and root disease severity index from bentazon-*Fusarium oxysporum* and bentazon/MCPA-*Fusarium oxysporum* treatments on red clover plants.
[a] Treatments: Control: untreated, F_0: *Fusarium* inoculum only, F_0/1X: Fo + Bentazon at 1X rate, F_0/2X: Fo + Bentazon at 2X rate. [b] Values, for each time period, followed by the same letter are not significantly different based on Conover-Inman's test ($P \leq 0.05$).

bentazon/MCPA had 25% and 100% increases in their RDSI, respectively, compared to the untreated control (Figure 1D).

Mycelial growth and conidia germination effects

Mycelial growth was slightly inhibited by both 1X and 2X rates of bentazon (Figure 2). The herbicide mixture bentazon /MCPA did not affect the mycelial growth with 1X rate, however, the 2X rate showed the strongest inhibition of *F. oxysporum* mycelial growth (Figure 2).

Conidial germination of *F. oxysporum* in a bentazon solution showed no significant differences compared to the control at all herbicide rates.

On the contrary, the germination of conidia was significantly reduced by bentazon/MCPA at the highest rate and a significant dose-response effect was observed; the suspended conidia in bentazon/MCPA solution at 1X and 2X rates had 28%, 33% and 40% reduction in germination, respectively, compared to the untreated control plants.

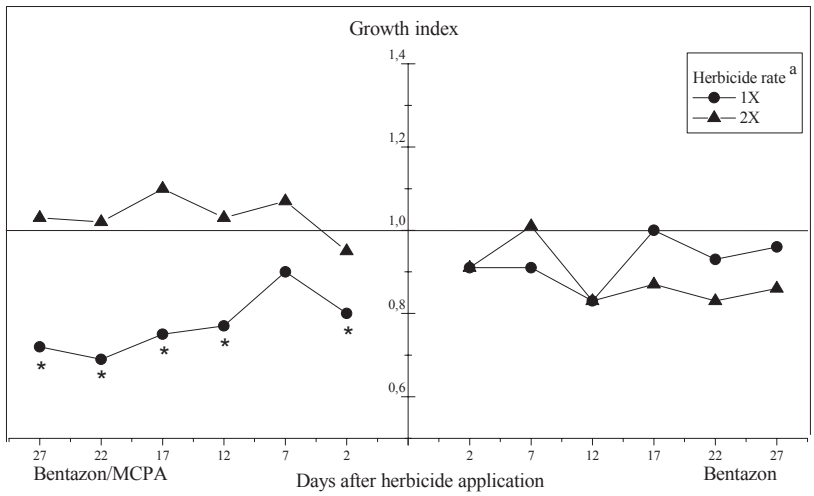

Figure 2: Mycelial growth of *Fusarium oxysporum* on agar-potato dextrose amended with bentazon/MCPA and bentazon. [a]Rate 1X: recommended rate; rate 2X: twice of recommended rate. *Indicates significant difference with corresponding control according to Dunnet-test ($P<0.05$).

DISCUSSION

In addition to the desired weed control, herbicides can result in a modification of disease development, generally as a result of the interaction between direct effects on the pathogen and indirect effects via plant-mediated responses (Altman and Rovira, 1989; Bollen, 1993; Liu *et al*., 1997, Sanogo *et al*., 2000). The range of herbicide concentrations used was selected to represent what would be encountered in production fields at the recommended rate (1X) and an accidental overlap of spray coverage (2X). The pathogen would be subjected to herbicide adsorbed directly from the spray solution and that from residues the plant or taken up and translocated from roots or foliage (Sanogo *et al*., 2000).

The present investigation showed that when the direct effect of bentazon and bentazon/MCPA on *F. oxysporum* was examined, bentazon had no effect on spore germination (Figure 3) and some, not significant, inhibitory effects on myceliar growth (Figure 2). On the other hand, bentazon/MCPA strongly inhibited spore germination and decreased mycelial development, at the highest rate. In our previous study of the effect of application of MCPA on *F. oxysporum*, herbicide alone had negligible effect on spore germination of *F. oxysporum* (Ceballos *et al*., 2006). Other studies with Fusarium spp. have also shown inhibitory responses with MCPA (Grossbard, 1976; Ceballos *et al*., 2006) but stimulation of *Fusarium* spp. growth by some herbicides such as has also been reported (Mussa and Rusell, 1977). Ceballos *et al*. (2006) in a greenhouse study with the same herbicide

rate showed that MCPA increased fusarium root rot severity to red clover seedlings. The effect of a herbicide on disease levels is not always the same as its effect on pathogen *in vitro* growth studies (Sanogo *et al*., 2000), because direct contact between the pathogen and the herbicide would not be as likely to occur under complex natural environmental conditions. Herbicide stress weakens and predisposes plants to rapid fungal colonization (Sanogo *et al.*, 2000; Ceballos *et al*., 2006), and could explain the significant increase in disease severity and pathogen isolation frequency following application of some herbicides (Dissanayake *et al*., 1998; Sanogo *et al*., 2000).

The influence of bentazon and bentazon/MCPA on infection by *F. oxysporum* on red clover has not been previously reported. Bentazon reduced the root dry weight and the bentazon/MCPA decreased crown diameter and shoot dry weight. Although comparisons could be made with other herbicides, where agronomic parameters were decreased (Ceballos *et al.*, 2006).

As in a previous study of the MCPA effect on fusarium infection (Ceballos *et al*., 2006), the application of bentazon and bentazon/MCPA resulted in a reduction of shoot growth and crown diameter. This topic is complicated by the intricate interactions among herbicide dose, formulation, tillage systems, environmental conditions, the plant pathogen, and the plant. Furthermore, the timing of infection with the pathogen vs. that of the herbicide treatment can have a profound influence on the interaction. Although the literature often appears to be conflicting, the apparent conflicts may be due to differences in one or more of the factors involved.

CONCLUSION

The effects of *F. oxysporum* inoculation and herbicide treatments seem be operating in different directions during the experimental period. *F. oxysporum* effect increases over time as disease progresses, and the effects of the herbicide may be reduced eventually as the plants outgrow the detrimental effects. However in this study, as in earlier investigations (Ceballos *et al*., 2004; Ceballos *et al*., 2006) the herbicide treatment in red clover plants infected with *F. oxysporum* increased plant disease. However these results necessarily must be validated with field experiments.

ACKNOWLEDGEMENT

The financial support of this research was provided by FONDECYT 1020297 and CONICYT PBCYT PSD05 Projects.

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