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EFFECTS OF POST-EMERGENCE HERBICIDES ON IN VITRO GROWTH OF FUSARIUM OXYSPORUM ISOLATED FROM RED CLOVER ROOT ROT

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ABSTRACT

In Chile, *Fusarium* root rot reduces red clover (*Trifolium pretense* L.) pasture yield and persistence. *Fusarium oxysporum* (Schlect.) is the most prevalent pathogen in diseased red clover plant roots. Agronomic management of red clover includes applying herbicides such as MCPA, 2,4-DB, flumetsulam, bentazon, and haloxyfop-methyl. In addition to weed control, herbicides can modify disease development, generally as a result of the interaction between direct effects on the pathogen and indirect effects via plant-mediated responses. The objective of this study was to evaluate the influence of these herbicides on *in vitro* growth of *F. oxysporum* at four application rates 0, 50, 100, and 200% at the field-recommended active ingredient rate. Herbicides were amended on Petri dishes containing potato dextrose agar (PDA) and buffer MUB (tris-hydroxymethyl-aminomethane). *Fusarium oxysporum* was cultivated at 22°C for 25 days and colony area was measured every 5 days. The herbicides MCPA and Flumetsulam had no effect on fungal growth. 2,4-DB showing an inverse dose effect on fungal growth varying between 16 and 35% at the end of the experimental period. The contact herbicide Bentazon exhibited the strongest inhibitory effect on *F. oxysporum* development by the application of the field recommended rate, with a 54% decrease with regard to the control at the end of the experiment. Haloxyfop-methyl showed the highest colony stimulation since 15 days after of the application, increasing 29% the colony area respect to the control at the end of the experiment. These results suggest that applying some herbicides to red clover could affect soil pathogens such as *Fusarium oxysporum*, increasing or inhibiting its development.

Keywords: phytopathogenic fungi, root diseases, mycelial growth.

INTRODUCTION

Red clover (*Trifolium pratense* L.) is an important perennial forage legume grown in southern Chile with 100 000 ha producing an average annual yield of 7 tons ha^{-1}. Although red clover is considered a perennial species, its yield declines each year and rarely persists more than 2 or 3 years. Root rot, caused by infestations of certain *Fusarium* species and root borer, *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae), are responsible for its short

persistence not only locally but worldwide (Steiner and Alderman, 1999). *Fusarium* root rot is a common disease in red clover pastures in Chile as well as in other areas in the world (Ceballos *et al.,* 2004, 2006; Steiner and Alderman, 1999), in which reduces productivity and persistence (Venuto *et al.,* 1999). *Fusarium oxysporum* Schldt. is the most commonly studied and economically important *Fusarium* species (Ceballos *et al.,* 2004; 2006; Venuto *et al.,* 1999).

Chemical weed control is currently a critical part of agricultural management to maximize red clover yield. The majority of postemergence herbicides presently used to protect red clover growth in Chile, and worldwide include MCPA ((4-chloro-2-methylphenoxy) acetic acid) and bentazon (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2 dioxide). Others, such as 2,4-DB (4-(2,4-dichlorophenoxy) butanoic acid), flumetsulam (N-(2,6-difluorophenyl)-5-methyl (1,2,4) triazolo (1,5-a) pyrimidine-2-sulfonamide), and haloxyfop-methyl (2-(4-(3-chloro-5-trifluoromethyl pyridyloxy) phenoxy) propionic acid) are less used (AFIPA, 2002; Kuds and Streibig, 2003). All these herbicides are described as highly selective. MCPA and 2.4-DB are systemic hormone-like herbicides absorbed by leaves, and also by roots as in the case of MCPA. Bentazon is a contact herbicide which acts directly where it is absorbed, primarily by foliage, but also by roots. Flumetsulam and haloxyfop-methyl are systemic herbicides absorbed by roots and leaves (Tomlin, 2003).

Besides affecting target weeds, herbicides can interact in several ways with plants and soil microorganisms that can influence the plant-pathogen relationship (Duke *et al*., 2007). Herbicides produce changes in the physiology and development of crop plants, thus increasing disease susceptibility. Herbicides can modify host plant structure and defense mechanisms which may lead to greater susceptibility to infection (Smiley and Wilkins, 1992). These changes include plant exudate modification and associated microorganism stimulation or inhibition (Mussa and Russel, 1977). In this context, herbicides are reported as affecting incidence and severity of plant diseases by interacting with plants, pathogens, or other microorganisms (Heydari and Misaghi, 2003). A number of plant diseases are reported to increase incidence and severity after applying herbicides (Duke *et al*., 2007; Sanogo *et al.,* 2000). Other diseases have shown either decreases or no significant changes (Dann *et al.,* 1999). No information is available on the consequences of non-target effects of these herbicides on *Fusarium oxysporum*. As such, the purpose of this research study was to evaluate the primary effects of selected herbicides on *Fusarium oxysporum* growth under *in vitro* conditions.

MATERIALS AND METHODS

Herbicides

This study was carried out with two independent experiments. Stock herbicide solutions were prepared by mixing commercial formulations in sterile water considering 200 L as the field application volume. The experiment consisted of the following herbicides: MCPA ((4-chloro-2-methylphenoxy) acetic acid), bentazon (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)- one-2,2 dioxide), 2,4-DB (4-(2,4-dichlorophenoxy) butanoic acid), flumetsulam (N-(2,6-difluorophenyl)-5-methyl (1,2,4) triazolo (1,5-a) pyrimidine-2-sulfonamide), and

haloxyfop-methyl (2-(4-(3-chloro-5-trifluoromethyl pyridyloxy) phenoxy) propionic acid). The assay was conducted under laboratory conditions and herbicides were applied at 0% (Control), 50% (0.5X), 100% (1X), and 200% (2X) which the rate applied by Chilean farmers for red clover (Table 1).

Table 1. Field-recommended active ingredient herbicide rate.

Herbicide	Commercial formulation [a]	Rate (g a.i. ha^{-1})	Rate / Dish (μg i.a.)
MCPA	MCPA 750 LS	750	47.7
2,4-DB	Venceweed 775 CE	388	24.7
Flumetsulam	Preside 80 G	39	2.5
Bentazon	Bentazon CS	1200	76.0
Haloxyfop-methyl	Galant Plus 30 CE	30	1.9

[a] CS: concentrate suspension; CE: concentrate emulsion; LS: liquid suspension; G: granules.

Fusarium isolate

Fusarium oxysporum isolates were collected in the Carillanca Experimental Station of the Instituto de Investigaciones Agropecuarias, Temuco, Chile and produced from diseased red clover plants with evident vascular wilt symptoms. Symptomatic root rot tissue was cut into 1-cm lengths, washed in distilled water, and soaked in a 1% NaOCl solution for 3 min (Akinsanmi *et al.,* 2004). A 1-cm subsection was cut from each sterilized section and placed on Petri dishes containing potato dextrose agar (39 g L^{-1} PDA, Difco) and streptomycin sulphate (100 mg L^{-1}). Dishes were incubated at 25º C for 14 d. Assays of *Fusarium* isolate pathogenicity s were performed with Quiñequeli red clover cultivar. The F127 isolate was identified as the most pathogenic, and was transferred to fresh PDA to complete Koch's postulates and continue the experiments (Ceballos *et al.,* 2006). The specific characterization of this strain is *F. oxysporum* (IMI 390980), and was provided by CABI Bioscience Identification Services, UK Centre (Egham).

Herbicide effects on *in vitro* mycelial growth

This study was carried out in optimal nutritional conditions for mycelial growth of *F. oxysporum* (Sanogo *et al.,* 2000). Potato dextrose agar (PDA, Difco) was amended with buffer MUB (tris-hydroxymethyl-aminomethane) to avoid pH modification by herbicide solutions. Commercial formulations of MCPA, haloxyfop-methyl, bentazon, flumetsulam and 2,4-DB were added to the agar dishes at the four rates previously described. A 5-mm diameter agar plug was collected from the edge of the actively growing F127colony (2-weekold culture), placed in the center of the herbicide-amended dishes (90 mm diameter), and incubated at 22°C in complete darkness.

Data analysis

Since colony growth was irregular, it was recorded by drawing its shape on a plastic film (Figure 1). The *F. oxysporum* colony area was calculated by scanning each plastic drawing with DIAS software. Growth was regularly recorded every 5 d

for 25 d, each treatment was replicated six times, and arranged in a completely randomized design. Area at the end of the experiment was compared by ANOVA, and group separation was performed with the Tukey test ($p \leq 0.05$) (Conover, 1999). Data were analyzed with StatsDirect statistical software, Version 2.7.2.

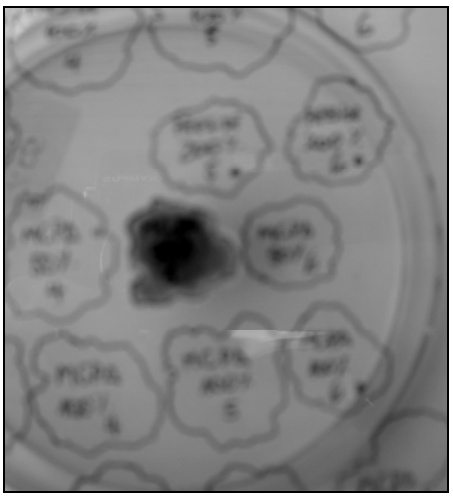

Figure 1. Plastic film to record irregular growth of *Fusarium oxysporum* colonies.

RESULTS AND DISCUSSION

The systemic herbicides MCPA and Flumetsulam did not show any statistical differences respect control throughout the experiment (Table 2). Bentazon, a type of contact herbicide, has no effect on fungal growth at the highest rate; though the *F. oxysporum* colony was significantly inhibited at the end of the assay with 54% at the field recommended rate (Table 2). *Fusarium oxysporum* growth in media amended with 2,4-DB decreased for all test rates (Table 2), 2,4-DB showed a significant inhibitory effect on fungal growth since 10 days after their application, and at the end of the experiment the detrimental effect was 35, 28, and 16% for 0.5X, 1X, and 2X rates, respectively (Table 2).

On the other hand, haloxyfop-methyl had a significant stimulant effect on fungus growth with the 2X rate, starting this effect at 15 days after their application (Table 2). By the end of the experiment, the colony was stimulated by 29% at the 2X rate (Table 2).

Stimulation of *Fusarium* spp. growth by some herbicides has also been reported (Mussa and Rusell, 1977). In this context, in a greenhouse study with the same herbicide rate, Ceballos *et al.* (2006) showed that MCPA increased the severity of *fusarium* root rot in red clover seedlings. Other studies with *Fusarium* spp. have also shown inhibitory responses (Grossbard, 1976). The variable responses of the herbicide fungi-toxic effect could be a result of the modification of the medium nutrient component with toxic substances from herbicide degradation (Liu *et al.,* 1997).

On the other hand, the effect of an 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 pathogen and herbicide would not be as likely to occur under complex natural environmental conditions. Herbicide stress weakens and predisposes plants to a rapid fungal colonization (Sanogo *et al.,* 2000; Ceballos *et al.,* 2006) and could explain the significant increase in disease severity and pathogen isolation frequency after applying some herbicides (Sanogo *et al.,* 2000).

Herbicide activity can be extended beyond its target organisms and its effects on various plant pathogens under laboratory conditions have been reported (Mussa and Russel, 1977; Heydari and Misaghi, 2003; Duke *et al.,* 2007; Ceballos *et al.,* 2009). In addition to weed

Table 2. *Fusarium oxysporum* growth (mm^2) in media amended with four rates of five herbicides.

Days after application	Proportion of the field-recommended rate			
	0	**0.5**	**1**	**2**
MCPA				
5	3.7 **ed**	2.5 **e**	3.2 **ed**	3.3 **ed**
10	13.1 **cd**	8.4 **ed**	11.4 **cde**	10.3 **cde**
15	20.5 **bc**	15.9 **bc**	20.7 **bc**	20.6 **bc**
20	27.4 **ab**	20.7 **bc**	26.8 **ab**	27.4 **ab**
25	31.8 **a**	25.2 **ab**	32.1 **a**	34.8 **a**
2,4-DB				
5	3.7 **hi**	2.4 **i**	3.0 **hi**	2.5 **i**
10	13.1 **g**	6.3 **hi**	7.1 **h**	6.0 **hi**
15	20.5 **def**	12.1 **g**	13.5 **g**	13.4 **g**
20	27.4 **b**	16.3 **fg**	18.2 **ef**	20.3 **def**
25	31.8 **a**	20.8 **de**	23.0 **cd**	26.8 **bc**
Flumetsulam				
5	3.7 **h**	2.9 **h**	3.0 **h**	3.1 **h**
10	13.1 **g**	9.8 **g**	9.8 **g**	12.0 **g**
15	20.5 **ef**	17.7 **g**	20.1 **ef**	21.2 **ef**
20	27.4 **bcd**	21.7 **ef**	24.2 **de**	26.4 **cd**
25	31.8 **ab**	28.0 **bcd**	30.8 **abc**	32.6 **a**
Haloxyfop-methyl				
5	3.7 **i**	3.1 **i**	3.7 **i**	4.7 **hi**
10	13.1 **fg**	12.3 **hg**	18.6 **efg**	16.9 **fg**
15	20.5 **def**	21.0 **def**	26.2 **c**	29.2 **bc**
20	27.4 **cd**	25.3 **cde**	30.1 **bc**	35.8 **ab**
25	31.8 **bc**	28.4 **c**	38.5 **ab**	41.1 **a**
Bentazon				
5	3.7 **d**	2.7 **d**	3.1 **d**	3.7 **d**
10	13.1 **cd**	9.0 **cd**	8.7 **cd**	11.8 **cd**
15	20.5 **abc**	15.6 **bcd**	10.7 **cd**	21.0 **abc**
20	27.4 **ab**	19.1 **abc**	12.8 **cd**	27.7 **ab**
25	31.8 **a**	22.5 **abc**	14.6 **bcd**	32.8 **a**

Means sharing a letter in common, for each herbicide, do not differ significantly according to Tukey test ($p \leq 0.05$).

control, herbicides can modify disease development, generally as a result of the interaction between direct effects on the pathogen and indirect effects via plant-mediated responses (Duke *et al.,* 2007; Sanogo *et al.,* 2000). The range of

herbicide concentrations was selected to represent actual production fields at the recommended rate (1X) and an accidental overlap of spray coverage (2X). The pathogen could adsorb herbicide directly from the spray solution or from plant residues that could be translocated from the roots or foliage (Sanogo *et al.,* 2000). This is a complex topic because of the intricate interactions among herbicide dose, formulation, tillage system, environmental conditions, pathogen, and the plant involved. Furthermore, the timing of infection with the pathogen *vs.* herbicide treatment can have a profound influence on the interaction. Although the literature often appears to be conflicting, apparent divergences may be due to differences in one or more of the factors involved. However, it is necessary that these results be validated with field experiments.

CONCLUSIONS

Overall our results suggest that various herbicides used to protect red clover against weeds could affect some root pathogen microorganisms, such as *Fusarium oxysporum.* The inhibition or stimulation of the fungal colony develop could be explain by mean of the products that occur in the modification of the medium nutrient and herbicide degradation by the fungus itself. It is clear that further studies are necessary since *Fusarium oxysporum* is the greatest cause of disease in red clover in Chile. Moreover, this research study was conducted under laboratory conditions and does not represent what could occur under field conditions where environmental factors influence the interactions between herbicide effects on *Fusarium oxysporum* and red clover.

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