EFFECT OF THE HERBICIDE FLUAZIFOP-BUTYL ON FUNGAL POPULATIONS AND ACTIVITY IN SOIL

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Abstract. The side effects of fluazifop-butyl on soil fungal populations and oxygen uptake were studied by incubating soil samples with a range of fluazifop-butyl concentrations (0, 0.6, 3 and 6 $\mu g\ g^{-1}$) over 8 weeks. Cellulose decomposition in soil was also studied in laboratory experiments with the herbicide which was either incorporated in soil or sprayed onto calico squares which were buried in soil. The mycelial dry weight of six fungal species under the effect of the herbicide was also examined. Fluazifop-butyl had no significant effect on total fungal propagule populations at 0.6 $\mu g\ g^{-1}$. At 3 and 6 $\mu g\ g^{-1}$, it caused temporary reduction in fungal populations observed after 1 and 2-wk of incubation. The herbicide had no significant effect on O_2 uptake. The decay of calico buried in herbicide-treated soil was generally stimulated, while the decomposition of herbicide-treated calico, buried in untreated soil, was temporary delayed. The mycelial dry weight yields of *Aspergillus flavus* (at 2 and 12 $\mu g\ mL^{-1}$ of fluazifop-butyl) and *Cunninghamella echinulata* (at 12 $\mu g\ mL^{-1}$) were significantly increased. At 24 $\mu g\ mL^{-1}$ the mycelial dry weight of *A. flavus* and *Alternaria alternata* was significantly reduced.

1. Introduction

Fluazifop-butyl is a member of phenoxy acid herbicides which are used because of their effectiveness against certain weed species. It is, then, not inconceivable that they may also influence certain of the microbial residents of soil. Because of the importance of fungi in soil systems, chemicals which interfere with the growth and activity of these organisms may influence nutrient cycling, energy flow, and other related fungal-mediated processes (Rosas and de Storani, 1987; Edwards, 1989). Extensive studies have been carried out on the side-effects of phenoxy acid herbicides application on soil microorganisms or microbial activities in soil (*e.g.* Grossbard, 1971; Ruffin, 1974; Marsh and Davies, 1978; Frioni, 1981; Schinner *et al.*, 1983; Magu and Bhowmik, 1984; Garabito *et al.*, 1991). However, there are few studies on the effect of fluazifop-butyl on microbial populations and activities in soil (*e.g.* Sapoundjieva and Kouzmanova, 1987). Therefore, the purpose of this study was to determine the side-effects of the herbicide fluazifop-butyl, when added to the soil, on the soil fungal populations. Oxygen uptake and cellulose decomposition in soil were selected as two parameters to detect the effect of the herbicide on soil microbial activity. Also, the effect of fluazifop-butyl on the mycelial dry weight of six soil fungal species was tested.

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2. Materials and Methods

2.1. Herbicide

The herbicide tested was fluazifop-butyl as "Fusilade": an aqueous solution of 25% active ingredient Butyl 2-[4-(5-trifluoromethyl)-2-pyridyloxy)phenoxy] propionate. Fluazifop-butyl was manufactured by Plant Protection Division, ICI, Fernhurst Haslemere, Surrey, England.

2.2. Soil treatment

A clay soil collected from Assiut area (Egypt) with a pH of 8.2, 0.7% total soluble salts and 2.0% organic matter contents, was used in the present investigation. The soil was screened through a 4 mm sieve. One kg aliquots of air-dried sieved soil were placed in polyethylene bags which were kept in a plastic pot. Fluazifop-butyl aqueous solution was sprayed and mixed throughout the soil to obtain four concentrations: 0 (control), 0.6 (equivalent to field dose), 3.0 (5-times field dose) and 6.0 (10-times) μg active ingredient per gram of dry soil while simultaneously rewetting the soil to 30% of its maximum water-holding capacity. Three replicates were used for each concentration. Pots were then incubated at 28 $\pm$ 1 °C for 8 weeks. After four incubation periods, i.e. 1, 2, 4 and 8 weeks following herbicide application, subsamples of each herbicide concentration were taken for assaying the counts of fungal propagules and measuring soil respiration.

2.3. Enumeration of fungal propagules

10 g (dry wt) of soil was removed from each soil subsample and used to provide a dilution series of 10^{-3}, 10^{-4} and 10^{-5} in 1% peptone solution. Fungal propagule counts were determined from the three dilution by using glucose-Cazpek's agar medium amended with 66 μg g^{-1} rose bengal.

2.4. Oxygen uptake

The oxygen uptake of the soil was measured manometrically with a Gilson respirometer. Aliquots of 4.5 mL soil suspension (5 g of control or treated soil in 20 ml sterilized distilled water) were placed in Warburg flasks. To absorb CO_2, the center well of each vessel contained 0.5 ml 10% KOH. Optimum oxygen consumption was measured at 30 °C for 2.5 hr period. Oxygen consumption was expressed as μL O_2 per g dry soil per h.

2.5. Decomposition of cellulose in soil

Squares of 5 $\times$ 5 cm^2 of calico (100% Egyptian cotton cloth) were used as a cellulosic substrate. One kg aliquots of air-dry sieved soil were placed in plastic pots.

TABLE I
Effect of fluazifop-butyl on soil fungal population

Herbicide dose	Propagules/g dry soil $\times 10^4$			
($\mu g\ g^{-1}$)	0	0.6	3.0	6.0
Time (wks)				
1	3.0	2.6	1.6[a]	1.7[a]
2	2.2	2.0	1.2[a]	1.4[a]
4	2.9	2.7	2.8	2.8
8	2.4	2.2	2.0	1.1[a]

[a] Significantly different from control at 0.05 level.

The herbicide was applied to either soil or calico at concentrations of 0, 0.6, 3.0 and 6.0 $\mu g\ g^{-1}$ on a dry soil basis. Decomposition of calico was studied as described by Greaves *et al.*, (1978). The calico was buried in the plastic pots with soil which were incubated at 28 ± 1 °C for 8 weeks. Four replicates of calico squares were prepared for each concentration. At four sampling times (1, 2, 4 and 8 weeks following herbicide treatment) the squares were removed, cleaned and loss in weight determined.

2.6. Pure culture experiment

Six soil fungal species isolated during the present investigation from herbicide-free soil were used in this study: *Aspergillus flavus* Link, *A. niger* van Tieghem, *Alternaria alternate* (Fr.) Keissler, *Cunninghamella echinulate* Thaxter ex Blakeslea, *Fusarium solani* (Martius) Sacchardo, and *Trichoderma harzianum* Rifai. These fungi were maintained on glucose-Czapek's agar medium. One ml heavy spore suspension (approx. 10^6 spores) of the required fungus was added to 250 mL Erlenmeyer flasks containing 50 mL culture medium. Fluazifop-butyl was added, asceptically, to the culture medium to give final concentrations 0, 2, 12 and 24 μg a.i. mL^{-1}. Four flasks were used for each concentration. Flasks were incubated at 28 ± 1 °C for 7 days. After incubation, cultures were filtered under suction and the mycelium produced was dried to constant weight at 80 °C.

2.7. Statistical analysis

Least significant difference analysis (LSD) was employed for statistical analysis of the results at 0.05% level.

TABLE II

Effect of fluazifop-butyl on soil respiration (as μL O_2 h^{-1} g^{-1} dry soil)

Herbicide dose (μg g^{-1})	0	0.6	3.0	6.0
Time (wks)				
1	0.25	0.26	0.39	0.30
2	0.29	0.31	0.22	0.23
4	0.80	0.83	0.66	0.50
8	0.34	0.55	0.39	0.36

3. Results

Addition of fluazifop-butyl to the soil at the rate of 0.6 μg g^{-1} did not cause a significant change to the soil fungal population during any of the incubation periods (Table I). At 3.0 and 6.0 μg g^{-1}, fluazifop-butyl appeared to decrease the fungal population after incubation for 1 and 2-weeks. This effect was completely alleviated after 4 and 8-weeks with 3.0 μg g^{-1}. However, fungal population counts were significantly reduced at the rate of 6.0 μg g^{-1} after 8-weeks incubation.

The results in Table II show that fluazifop-butyl did not cause any significant effect on oxygen uptake by soil at any dose used.

Results in Table III show the effect of fluazifop-butyl on cellulose decomposition when applied either to calico or soil. The decay of calico buried in the herbicide treated soil was generally stimulated at all sampling periods. This effect was significant only after 1 wk (at 3.0 and 6.0 μg g^{-1}) and 4 wk (at the three doses used). In contrast, application of the herbicide to the cellulosic substrate which subsequently is buried in untreated soil caused significant reduction in weight loss of the calico in the first period of incubation (1 wk). This effect disappeared after the longer periods of incubation.

The effect of fluazifop-butyl on the mycelial dry weight of test fungi is presented in Table IV). A concentration of 2 μg mL^{-1} of fluazifop-butyl did not significantly affect the mycelial growth of any of the test fungi, except *Aspergillus flavus* which was stimulated. The dry weight yields of *A. niger* and *Cunninghamella echinulata* were significantly increased at 12 μg mL^{-1} of fluazifop-butyl. On the other hand, the herbicide reduced the dry weight gain by *A. flavus* and *Alternaria alternata* at a concentration of 24 μg mL^{-1}.

TABLE III

Effect of fluazifop-butyl on the weight loss of calico buried in soil (% weight loss)

Incubation time (wks)	Herbicide concentration $\mu g\ g^{-1}$	Soil treated, calico untreated	Calico treated, soil untreated
1	0.0	3.8	3.8
	0.6	5.0	5.3
	3.0	11.2[a]	0.0[a]
	6.0	11.6[a]	0.0[a]
2	0.0	16.5	16.5
	0.6	20.8	17.8
	3.0	25.0	8.6
	6.0	27.7	6.4
4	0.0	31.4	31.4
	0.6	38.3[a]	37.3
	3.0	49.9[a]	37.7
	6.0	44.2[a]	26.6
8	0.0	48.5	48.5
	0.6	55.1	49.9
	3.0	59.0	45.0
	6.0	63.1	50.6

[a] Significantly different from the control.

TABLE IV

Effect of fluazifop-butyl on dry weight yield (calculated as a percentage of the control) of test fungi

Species	Herbicide concentrations $\mu g\ g^{-1}$		
	2	12	24
Aspergillus flavus	115[a]	118[a]	84[a]
A. niger	108	113	119
Alternaria alternata	94	85	67[a]
Cunninghamella echinulata	105	147[a]	80
Trichoderma harzianum	116	122	199

[a] Significantly different from the control.

4. Discussion

From the data reported here, it is evident that fluazifop-butyl gives no significant change in fungal population counts at the lower concentration (0.6 μg g^{-1}). Temporary inhibition in fungal counts was observed at the higher rates (3.0 and 6.0 μg g^{-1}). For comparison other phenoxy acid herbicides like, 2,4-D, MCPSA and 2,4,5-T neither have any adverse effect on the total numbers of microorganisms (Fletcher, 1960; Frioni, 1981; Magu and Bhowmik, 1984). However, the involvement of soil microorganisms in the degradation of phenoxy acid herbicides in the soil environment was demonstrated by several workers (e.g. Audus, 1952, 1964; Loos, 1969).

In an investigation by Sapoundjieva and Kouzmanova (1987) the effect of fluazifop-butyl on fungi depended on the application rate, 4 L ha^{-1} stimulating their growth. Garabito *et al.* (1991) reported that soil microbial populations tend to normality around 6 weeks after exposure to 2,4-D.

In this investigation, oxygen uptake by soil treated with fluazifop-butyl was not significantly affected at any of the doses used. In this respect, Ruffin (1974) found no effect of 2.4-D or 2,4,5-T on oxygen uptake in two different soils. However, inhibition in O_2 uptake by 100 ppm of 2,3,5-T in Triangle soil during the second half of incubation was observed by Marsh and Davies (1978).

The results obtained during the present investigation indicate that the effects of fluazifop-butyl on calico decomposition in soil varied from stimulation or inactivity to inhibition. The effect depends on the mode of application of the herbicide. In this respect, the phenoxy acid herbicides have no effect even at high concentration on the decomposition of cellulose by *Trichoderma viride* in soil and *in vitro* (Ramanujan *et al.*, 1978).

In the pure culture experiment, the fungi responded variously to different concentrations of the herbicide. The mycelial dry weight yields of two of them were not significantly affected at any of the doses (*A. niger* and *T. harzianum*). Both *A. flavus* and *Alt. alternata* were reduced at 24 μg mL^{-1}). On the other hand, *A. flavus* (at 12 μg mL^{-1}) and *C. echinulata* (at 24 μg mL^{-1}) were significantly stimulated. Many studies have established that phenoxy acid herbicides can be degraded by pure cultures of microorganisms. This capability has been identified in several genera of bacteria as well as in fungi and actinomycetes (Loos, 1969; Evans *et al.*, 1971). However, the response of fungi to herbicides in pure culture may not accurately reflect their response to the same chemicals during field conditions (Anon, 1987; Greaves, 1987; Wardle and Parkinson, 1990).

From the results of this investigation it is concluded that the herbicide fluazifop-butyl when used on the soil type studied, in field dose, will not have any particular effect, either on fungal population counts or on other microbial activities tested.

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