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Nontarget effects of the herbicide tebuthiuron on mycorrhizal fungi in sagebrush semidesert

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Abstract. The effects of the herbicide tebuthiuron (0.36, 0.6, and 1.01 kg/ha in pellet form) on nontarget organisms, vesicular-arbuscular mycorrhizal fungi, were observed in sagebrush semidesert in central Utah. Only the highest level of tebuthiuron application showed any significant effects on mycorrhizal fungi compared to the untreated control. The introduced annual *Bromus tectorum* L. had both a reduced percent mycorrhizal root infection and reduced spore density in its rhizosphere with the highest herbicide level. The herbicide did not significantly affect mycorrhizal root infection of *Sitanion hystrix*, a short-lived perennial grass, at any level of application. There was no significant effect of any level of tebuthiuron on germination of mycorrhizal spores collected 6 months after herbicide application.

Key words: Herbicide – Vesicular-arbuscular mycorrhizal fungi – *Bromus tectorum* – *Sitanion hystrix*

Introduction

Herbicides are often used to control undesirable dicotyledonous plants in pastures and rangelands, but some pesticides such as fungicides and soil fumigants also reduce the abundance of vesicular-arbuscular (VA) mycorrhizal fungi (Menge 1982). However, little is known about the effects of herbicides on these nontarget organisms. For instance, paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) and napropamide (*N*,*N*-diethyl-2-(1-naphthalenyloxy)-propionamide) reduced the efficiency of the VA mycorrhizal fungus *Glomus fasciculatum* (Thaxter sensu Gerdemann) Gerdemann and Trappe in *Fraxinus americana* L. (Pope and Holt 1981). Chlortoluron (3-(3-chloro-*p*-tolyl)1,1-dimethylurea) reduced mycorrhizal infection of crop plants but also increased soil spore density (Ocampo and Hayman 1980). VA mycorrhizae improve the growth of many rangeland grass species by increasing biomass, increasing photosynthetic and transpiration rates, increasing nutrient uptake, and improving drought stress tolerance and water use efficiency (Allen et al. 1981a, b; Wallace 1981; Allen and Boosalis 1983; Dickman et al. 1984; Allen and Allen 1986; Miller et al. 1987; Di and Allen 1991). Thus mycorrhizae may play a key role in rangeland productivity (Trappe 1981), and studies of the effects of herbicides on mycorrhizae are needed.

Tebuthiuron (*N*-(5-(1,1 dimethylethyl)-1,3,4-thiadiazol-2-yl-*N*,*N*′-dimethylurea) is used to control woody plant abundance for increased forage yield on western rangelands (Britton and Sneva 1984; Clary et al. 1985; McDaniel and Balliette 1986). However, the herbicide may affect nontarget plants (Bovey et al. 1982; Masters and Scifres 1984; Whisenant and Clary 1987). A reduction in nontarget plants may be a direct effect of the herbicide, but it may also be indirect if it reduces the incidence of VA mycorrhizal fungi. Sagebrush (*Artemisia tridentata* Nutt.) is often controlled by herbicides to increase grass forage in rangelands of the western United States (e.g., Whisenant and Clary 1987). We examined the effects of tebuthiuron on VA spore numbers and germination, and percent mycorrhizal infection of the dominant grasses after tebuthiuron application in sagebrush semidesert in central Utah.

Materials and methods

The study site was near Tintic Junction, Juab County, in central Utah at a research site cooperatively maintained by Utah State University and the Bureau of Land Management. The site has been degraded by heavy cattle grazing. The vegetation at the site is dominated by *A. tridentata* ssp. *tridentata* with an understory of sparse native perennial grasses and an abundance of the introduced annual *Bromus tectorum* L. (Reynaga-Valdes 1983; West et al. 1984). The soil is a coarse-loamy, mixed, mesic, Torrifluventic Haploxeroll (1–3%). The annual precipitation is 360 mm (20-year mean) with a peak in the autumn and winter. Precipitation during the September 1981–August 1982 study period was 381 mm, with October 1981 being the wettest month during this 12-month period

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(56 mm). Autumn precipitation generally initiates germination of *B. tectorum* (Ganskopp and Bedell 1979), as it did during the autumn of this study.

Pelleted tebuthiuron was applied at three levels on two sampling blocks on 5 October 1981. The herbicide (20% a.i. pellets with dimensions of 32×50 mm) was broadcast using a tractor-mounted cyclone seeder at rates of 0.36, 0.60, and 1.01 kg/ha a.i. on 35×75 m plots. An untreated control plot was also maintained in each block.

The effectiveness of the herbicide for sagebrush control and effects on nontarget plants were evaluated at this site during 1982 (Reynaga-Valdez 1983). Phytomass of sagebrush was reduced by 94, 93, and 98% 1 year after application of 0.36, 0.60, and 1.01 kg/ha tebuthiuron, respectively. The density of *B. tectorum* in spring 1982 was significantly reduced only by the highest level of application, from 20 individuals/m^2 in the control to 10/m^2. The average cover of perennial grasses, dominated by *Sitanion hystrix* (Nutt.) J. G. Smith, on this degraded rangeland was only about 1%, and was not significantly reduced by tebuthiuron in the spring of 1982 (Reynaga-Valdez 1983).

Soils and roots were sampled for VA mycorrhizal fungi on 22 April and 21 June, 1982. Soil cores (6 cm dia×10 cm) were collected at random within the rhizospheres of the two most abundant grasses, *B. tectorum* and *S. hystrix*. Three to five cores were collected for each of the two grass species in each tebuthiuron treatment and control plot. Both grass species were sampled in June, but there was little active growth of *S. hystrix* in April and it could not be sampled. The cores were stored frozen for several months until they were analyzed.

After thawing, the rhizosphere soil was shaken loose from the *B. tectorum* samples for spore counts and germination. Spores were separated from 5 g of homogenized rhizosphere soil from each core using sucrose flotation (Allen et al. 1979). Spores were counted under 40× magnification and values were expressed as number of spores per g dry soil. After counting, the spores were placed in water at room temperature for 10 days and allowed to germinate (Warner et al. 1987). Roots of *B. tectorum* and *S. hystrix* were washed and stained with trypan blue (Kormanik et al. 1980) to determine the percent root infection. Only those roots that were attached to the plant were used to assure that roots of no neighboring species were sampled. Fifty 1-mm root segments of each sample were observed under 100× magnification for the presence or absence of arbuscules, vesicles, or hyphae, and values were expressed as percent of root segments infected with mycorrhizal fungi.

The data were subjected to one-way analyses of variance followed by tests of the least significant difference at $P<0.05$ ($LSD_{0.05}$)

Results

The treatment with 1.01 kg/ha tebuthiuron significantly reduced the percent mycorrhizal root infection of *B. tectorum* compared to the control and 0.36 level, but it was not significantly lower than the 0.6 kg/ha treatment in April (Table 1). There were no significant differences in root infection of *B. tectorum* between any of the herbicide levels and the control in June. Tebuthiuron also did not significantly reduce root infection of *S. hystrix* in June, which could not be sampled in April because it was not mature enough (Table 1).

Tebuthiuron did not reduce spore density in the rhizosphere of *B. tectorum* in April, but in June spore density was significantly lower in the 1.01 level than the control (Table 2). Germination of spores from the rhizosphere of *B. tectorum* was not significantly affected by tebuthiuron in April or June at any application level (Table 2).

Discussion

Because VA mycorrhizal fungi are obligate symbionts, practices that remove host plants, such as application of herbicides, will eventually reduce the inoculum density of the fungi if new plant growth is not initiated soon after the application. In this short-term study, autumn application of tebuthiuron caused significant reductions

Table 1. The effects of three concentrations of tebuthiuron on percent mycorrhizal root infection of *Bromus tectorum* and *Sitanion hystrix*. * Significant at $P \leq 0.05$, NS, not significant. Data are percent infection

Species	Month	Tebuthiuron (kg/ha)				
		0	0.36	0.60	1.01	$LSD_{0.05}$
Bromus	April	22.5*	22.7*	12.0*	10.8*	11.0*
	June	8.0 (NS)	2.7 (NS)	8.0 (NS)	1.3 (NS)	13.0 (NS)
Sitanion	June	18.0 (NS)	12.0 (NS)	24.0 (NS)	9.3 (NS)	22.5 (NS)

Table 2. The effects of three concentrations of tebuthiuron on spore density and spore germination in rhizosphere soil from *B. tectorum*. * Significant at $P \leq 0.05$, NS, not significant

Month	Tebuthiuron (kg/ha)				
	0	0.36	0.60	1.01	$LSD_{0.05}$
Spores/g soil					
April	118.3 (NS)	59.5 (NS)	103.9 (NS)	74.2 (NS)	105.0 (NS)
June	57.5*	43.6*	19.2*	5.6*	50.2*
Spore germination (%)					
April	4.1 (NS)	3.3 (NS)	2.0 (NS)	2.5 (NS)	4.9 (NS)
June	12.7 (NS)	5.2 (NS)	6.2 (NS)	8.9 (NS)	8.5 (NS)

in percent root infection and spore density in the following spring only under the highest application rate, 1.01 kg/ha. The recommended rate of application is 0.6 kg/ha (Reynaga-Valdez 1983; MacDaniel and Balliette 1986). The herbicide is unlikely to continue to affect the fungi, as it degraded at an exponential rate in the same study area in 1984–85, years with 30% above normal precipitation (Whisenant and Clary 1987). The unusually high precipitation during the study period ensured the rapid dissolution of the tebuthiuron pellets (Van Pelt and West 1989) and movement of the herbicide into the rooting zone (Whisenant and Clary 1987).

The two host plants also form VA mycorrhizae at other sites, but the fungi have various effects on their growth. *S. hystrix* had 14% increased biomass with low mycorrhizal infection (Fransen and Miller 1985). Infection percentages of *B. tectorum* as high as 76% have been reported (Bethlenfalvay and Dakessian 1984), but mycorrhizal infection did not increase the biomass of *B. tectorum* in two studies (Allen 1984; Schwab and Loomis 1987). This implies that the reduced percent infection of *B. tectorum* at the 1.01 kg/ha application rate is unlikely to explain the reduction in its abundance, so a direct negative effect of the herbicide on the grass is the more likely hypothesis (Reynalda-Valdez 1983).

The phenology of the host plant and fungus may explain why tebuthiuron did not significantly reduce the mycorrhizal infection of *S. hystrix* while it did for *B. tectorum*. The phenology of the fungus is tightly coupled to that of its host, and the fungus is most active at times of rapid plant growth (Allen 1983; Allen et al. 1984). *B. tectorum* is a winter annual which germinated during the autumn when tebuthiuron was applied. Mycorrhizal infection of *B. tectorum* was also observed in the autumn (Allen, unpublished observation). The senescence of *B. tectorum* at the highest application rate (Reynaga-Valdes 1983) correlates with the reduced infection in this treatment in April. By June, when *B. tectorum* had produced seed and was nearly dormant, no significant effect of the herbicide could be detected on the mycorrhizal fungi. *S. hystrix*, by contrast, is a summer perennial that was dormant during the autumn of application, and most of the herbicide had probably already broken down when sampling occurred the following spring (Whisenant and Clary 1987). Thus the herbicide did not affect either plant abundance or percent mycorrhizal root infection of *S. hystrix*. The low abundance of *S. hystrix* may in addition have made detection of a significant effect unlikely.

The effect of tebuthiuron on spore density in the rhizosphere of *B. tectorum* in June but not April might also be due to phenology. Mycorrhizal fungi of *B. tectorum* produce spores primarily in the spring, just before the annual completes its life cycle. The spores that were sampled in April 1982, therefore, were likely produced during the previous growing season, before tebuthiuron was applied. The new spores produced in June were from plants and fungi that had been impacted by tebuthiuron applied at the highest rate. Even though spore production was reduced after tebuthiuron application, spore germination was not significantly affected either for those spores that overwintered in the soil (April samples) or for those that were newly produced in spring 1982 (June samples).

Tebuthiuron applied in this sagebrush semidesert reduced percent infection and spore densities of surviving grasses only at the highest application rate (1.01 kg/ha), and this occured only for *B. tectorum*, the species that was actively growing during and immediately after the time of application. Although viable spores persisted in the soil even at the highest application rate, the most important source of mycorrhizal infection for new roots is thought to be via hyphal contact from established roots (Read 1992). Any practice that removes the dominant vegetation, such as mortality of *A. tridentata* in this study, will also necessarily decrease the carbon source for hyphal growth and future spore production of these obligate symbionts, and necessitates the rapid re-establishment of new vegetation, either by natural succession or seeding, for survival of the inoculum.

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