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EFFECTS OF WILLOWS (*Salix brachycarpa*) ON POPULATIONS OF SALICYLATE-MINERALIZING MICROORGANISMS IN ALPINE SOILS

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Abstract—We used the substrate-induced growth-response (SIGR) method to quantify salicylate-mineralizing microbes and total microbial biomass in soils from under willows (*Salix brachycarpa*) and in surrounding meadows dominated by the sedge *Kobresia myosuroides*. Willows had a strong effect on the biomass of salicylate-mineralizing microbes in both years of this study. There were always higher biomass levels of salicylate mineralizers in soils from under *Salix* (4.6–10.1 μg C/g) than under *Kobresia* (0.23–0.76 μg/g). In contrast, total microbial biomass was not significantly different under these plant species in 1996 and was only higher under *Salix* on one date in 1997. These results show that the standing biomass and activity of salicylate-mineralizing microbes can be greatly enhanced by salicylate-producing plants in the field. Given this finding, it is unlikely that simple phenolic compounds like salicylate would persist for very long in soil beneath the plants that produce them.

Key Words—Allelopathy, alpine soils, salicylate, *Salix*, SIGR.

INTRODUCTION

Understanding the distribution of microorganisms relative to plant distribution is important to understanding how plants influence soil processes and neighboring plants. Plants support heterotrophic microbial biomass via root exudation and through organic matter input in the form of litter and dead roots. Therefore, it is not too surprising that the distribution of plants has been shown to affect the spatial distribution of heterotrophic microbes in nature (Herman et al., 1995; Smith et al., 1994; Wardle, 1992).

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Little is known, however, about how plants influence the spatial distribution of specific microbial groups in nature. There is some evidence that plants that produce specific phenolic compounds may select for populations of soil microbes that metabolize and detoxify such compounds (Schmidt, 1990; Schmidt and Ley, 1999). If this is true, then there should be higher populations of phenolic-metabolizing organisms under plants that produce such compounds than under non-producing plants in the same soil. We tested this hypothesis by quantifying the biomass of salicylate-mineralizing microbes under a salicylate producer (*Salix brachycarpa*) and in adjacent soil dominated by the alpine sedge *Kobresia myosuroides*.

METHODS AND MATERIALS

Soil Sampling. We sampled soils from beneath willows (*Salix brachycarpa* Nuttall) and in *Kobresia myosuroides* (Vill.) Fiori & Paol. meadows that surround the willows. The sampling sites range in elevation from 3475 to 3550 m (40°03′N, 105°35′W) and are all located to the east of the long-term ecological research (LTER) grid on Niwot Ridge, Colorado, USA (Fisk and Schmidt, 1995). Our sites are fairly uniform with regard to soil type and topography, and the soil properties of these sites have been described in detail elsewhere (Holtmeier and Broll, 1992; Marr, 1977; Pauker and Seastedt, 1996). Soils were sampled to a depth of 10 cm with a soil corer (2.5 cm diam.). Because we were limited as to the amount of soil that we could remove from these sensitive high-elevation sites, soils from under at least three randomly chosen willows were pooled at each sampling date. Extra sampling dates were added in the second year of the study so that statistical comparisons of the data would be more robust. Soils were kept at 4°C until they were processed in the laboratory.

Biomass Measurements. Biomass or population levels of different microbial functional groups were estimated by the substrate-induced growth-response (SIGR) method (Schmidt, 1992), which has been validated in recent studies of alpine tundra (Lipson et al., 1999a,b). Soils (10 g dry wt equivalent) were incubated in biometer flasks (Schmidt and Scow, 1996) with substrates added at concentrations that were previously determined to induce growth of specific microbial functional groups. The concentration of salicylate was 200 μg C/g, whereas 2000 μg C/g of glucose or glutamate were used. In addition, a small amount of ^{14}C-labeled substrate (<0.1 μCi/flask) was also added as a tracer of CO_2 production from the substrate. Carbon dioxide emitted from the soil was captured in 0.5 N NaOH in the sidearm of the biometer flask. At regular intervals, samples were removed with a syringe and replaced with fresh NaOH solution. Growth of the biomass of a particular functional group was evident if the rate of $^{14}CO_2$ evolution increased over time. The increase in the rate of mineralization was used to estimate both the intial biomass level and the maximum specific growth

rate of that biomass by fitting the SIGR equation (Colores et al., 1996) to the data:

$$dP/dt = \mu_{\max} X_1 e^{\mu_{\max} t} \quad (1)$$

in which X_1 is microbial biomass in terms of CO_2 produced, P is CO_2 carbon and $\mu_{\max}$ is the maximum specific growth rate of the microbial functional group. To convert X_1 to X_0 (with units of micrograms of biomass C per gram of soil), we used the relationship of Colores et al. (1996):

$$X_0 = X_1(Y_c/1 - Y_c) \quad (2)$$

where Y_c is the yield coefficient with units of micrograms biomass C/micrograms substrate C. Thus, X_0 is an estimate of the biomass of soil microbes that can mineralize a specific substrate in soil.

Salicylate Analyses. Plant materials collected mid-August 1998 at Niwot Ridge (50 mg equivalent dry weight) were ground to a fine powder in liquid N_2 in a ceramic mortar and pestle. Subsamples were removed immediately and dried at 100°C for 48–72 hr for fresh weight/dry weight conversion. The powder was extracted with an initial 3 ml of 50% methanol–water (HPLC grade), and the mortar rinsed with an additional 2 ml of 50% methanol. The resulting extract was centrifuged at 5000*g*, and the liquid decanted for analysis. Samples were analyzed on a Beckman System Gold HPLC, with aromatic acids eluted by isocratic 60% aqueous 50 mM KH_2PO_4 (pH 3.2): 40% methanol at 1 ml/min on a model 128 pump. The column was maintained at 30°C in a column heater with a model 507 autosampler, and separated on a C_{18} reversed-phase column (150 cm × 4.6 mm; 5 mm pore size; Jones Chromatography). Salicylic acid eluted at 5.8 min and was detected at 254 nm on a model 166 detector. Aliquots of the methanol extracts were also treated with 3 N HCl, heated at 35°C for 30 min, and separated as before. Operationally, this fraction represented salicylates with glycosidic linkages (Lindroth and Pajutee, 1987), based on the behavior and recovery of authentic standards of salicyclic acid (Na salt; Sigma-Aldrich Co.) and salicin prepared in 50% methanol. By the method of standard recoveries, acid hydrolysis led to ~80% recovery of salicin standards, so no correction was applied. There may be additional salicylate compounds in the tissues, but the purified compounds are not available commercially.

RESULTS

Some *Salix* species have high levels of salicylates, whereas others do not produce measurable quantities (Julkunen-Titto, 1989; Lindroth and Pajutee,

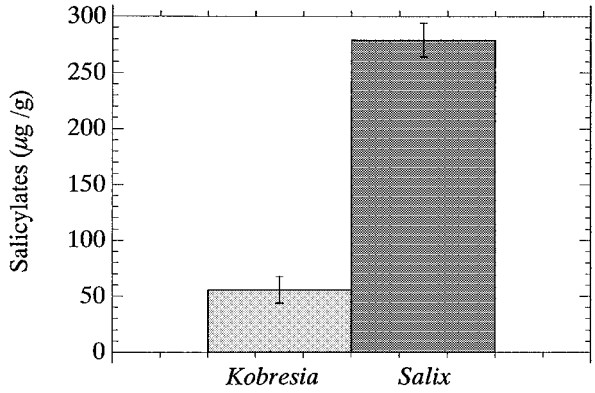

FIG. 1. Concentrations of salicylates in stems of *K. myosuroides* and bark of *S. brachycarpa* collected on August 13, 1998.

1987). We therefore needed to determine if *S. brachycarpa* was a salicylate producer. Figure 1 shows the levels of salicylates in bark of *S. brachycarpa*. As a control, we also measured salicylate levels in the stems of *K. myosuroides* collected on the same date (Figure 1).

Preliminary studies were done in July 1996 to determine if the SIGR method could be used to quantify the biomass of salicylate-mineralizing microbes under different plant species. The estimated biomass of salicylate mineralizers was much higher in soils from under willows than in soils from under *K. myosuroides* (Figure 2). To determine if more generalist populations of microbes were simi-

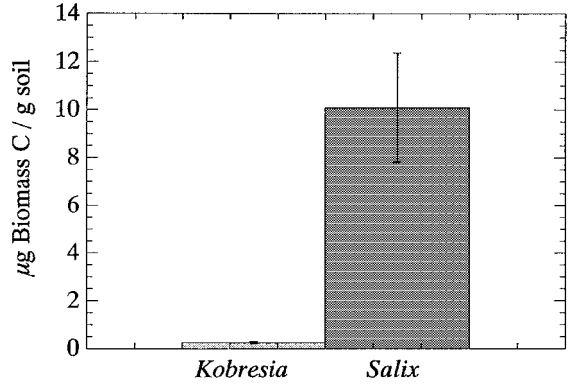

FIG. 2. Biomass estimates for salicylate-mineralizing microbes in *S. brachycarpa* and *K. myosuroides* soils collected on July 25, 1996.

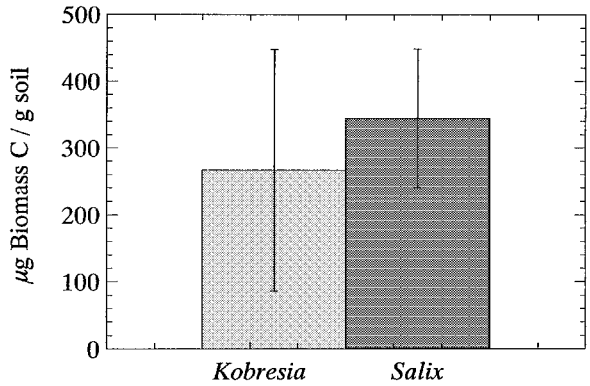

FIG. 3. Total microbial biomass estimates in *S. brachycarpa* and *K. myosuroides* soils collected on July 25, 1996.

larly affected by plant species, we carried out SIGR analyses with glucose as the substrate. Results from these incubations are shown in Figure 3. Total microbial biomass was high in both soils, and there were no statistical differences between the soils.

Given the above results, we expanded our studies in 1997 by increasing the sample sizes. The spatial pattern of salicylate mineralizers held up for the three sampling dates in 1997 (Figure 4). Over the two years of this study, biomass of salicylate mineralizers under willows ranged from 4.6 to 10.1 μg C/g compared to 0.23–0.76 μg C/g in *K. myosuroides* soils. When all of the salicylate-

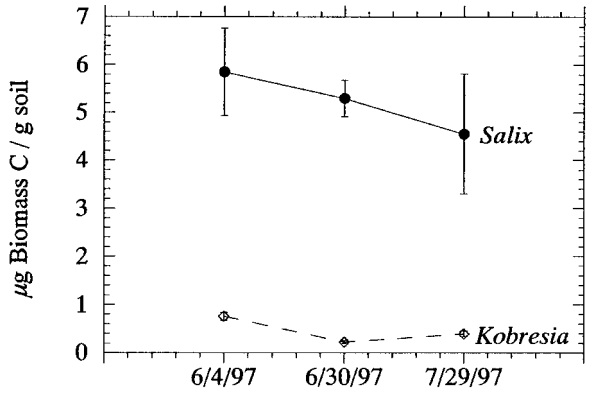

FIG. 4. SIGR biomass estimates for salicylate-mineralizing microbes in *S. brachycarpa* and *K. myosuroides* soils collected on three dates in 1997.

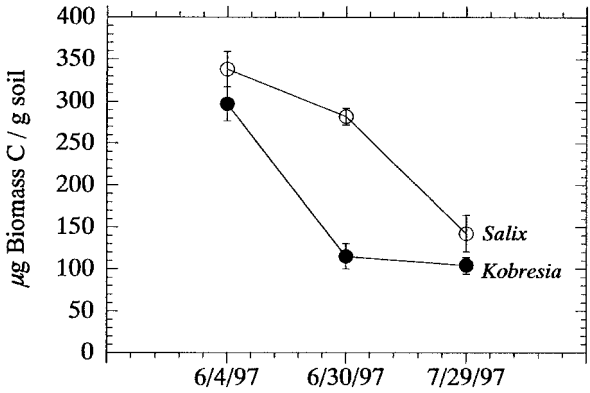

FIG. 5. Total biomass estimates for glutamate-mineralizing microbes in *S. brachycarpa* and *K. myosuroides* soils collected on three dates in 1997.

mineralizer data for both years were analyzed by a two-way ANOVA, it showed that there were more salicylate mineralizers in the *S. brachycarpa* soils than the *K. myosuroides* soils ($P < 0.001$). In contrast, total microbial biomass was only higher under *S. brachycarpa* than under *K. myosuroides* on one date in 1997 (Figure 5).

DISCUSSION

The main purpose of this study was to determine if the biomass of specific microbial functional groups can be controlled by plant species. Specifically, we tested whether populations of salicylate-mineralizing microbes are different among soils from under the two dominant plant species at our study sites. Our results show that there is a much higher population of salicylate mineralizers under *S. brachycarpa* than under *K. myosuroides*. This study also shows that this "willow effect" was not due to a generalized increase in microbial biomass under willows, as there was not always a higher total microbial biomass under willows. The simplest explanation for our results is that the willows produce more salicylate than *K. myosuroides* and that the excess salicylates under willows support higher populations of salicylate-mineralizing microbes.

To our knowledge, this is the first study that has attempted to estimate the biomass of a specific microbial functional group (salicylate mineralizers) in the soil beneath specific types of plants. Most similar studies have been done to measure the total microbial biomass under different plant species. For example, Herman et al. (1995) showed that total heterotrophic microbes were more abundant under desert shrubs than in the soil between shrubs. Likewise, Smith

et al. (1994) found higher microbial biomass levels and respiration rates under *Artemesia tridentata* shrubs than in soil between the shrubs. The uneven distribution of bacteria in dry-shrub and desert systems probably results from the patchy distribution of resources in these systems. Nutrients and water are usually more abundant under dry-land shrubs than between them (Burke et al., 1989; Charley and West, 1975; Herman et al., 1995; Smith et al., 1994). At our sites in the alpine area of Colorado, soil nutrients are less patchy in a given plant community (Fisk et al., 1998), and soil organic matter levels are high both under and between willows. Thus, it is not too surprising that our estimates of total heterotrophic biomass were not always higher under willows.

Our results have implications for the fate and effects of some phenolic compounds produced by plants. Our results indicate that it is likely that any plant that produces copious amounts of a compound will select for high populations of microbes that metabolize that compound. Therefore, it seems unlikely that compounds such as salicylate would move very far in soil before being metabolized. Salicylate has been implicated as an allelopathic chemical based on laboratory bioassays (Harper and Balke, 1981; Leslie and Romani, 1988; reviewed in Pierpoint, 1994). However, given the large biomass of salicylate-mineralizing microbes in soil from under willows and the efficient scavenging ability of soil microbes (Schmidt and Ley, 1999), it seems unlikely that salicylate would build up to phytotoxic levels under willows in the field. In preliminary, pool-labeling experiments we found that levels of salicylate were below 0.1 μg/g in soils collected on July 29, 1997 from beneath willows and *K. myosuroides* plants (Schmidt and Lipson, unpublished data). More work is needed, however, before we can conclude that salicylate does not build up and that large populations of salicylate mineralizers persist year round under willows.

Finally, our results may also have broad applicability to studies of the fate of phenolic compounds that are much more toxic than salicylate. Some of the most toxic phenolic compounds known are pentachlorophenol (PCP) and 2,4-dinitrophenol (DNP). We have observed buildups of specified microbial populations in response to applications of these compounds to soil (Colores and Schmidt, 1999; Schmidt and Gier, 1989). For example, when DNP was applied to soil at the same concentration as salicylate (200 μg/g), it caused a dramatic buildup of DNP-mineralizing microbes even in soil that had never been exposed to DNP (Schmidt and Gier, 1989). In addition, we have observed long-term (several months) survival of DNP- and PCP-mineralizing populations in soils exposed to environmentally relevant concentrations (up to 300 μg/g) of these chemicals (Colores and Schmidt, 1999; Schmidt and Gier, 1989). Thus, it seems likely that even very toxic plant-produced phenolic compounds would have positive effects on phenolic-mineralizing populations in soil, making it unlikely that phenolic compounds would persist in soils that are chronically exposed to such compounds.

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