J. Range Manage.
55: 420-423 July 2002

Fingerprint composition of seedling root exudates of selected grasses

JOHAN F. DORMAAR, BONNIE C. TOVELL, AND WALTER D. WILLMS

Authors are Soil Scientist (retired), Microbiologist, and Range Ecologist, Research Centre, Agriculture and Agri-Food Canada, P. O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1

Abstract

The competitiveness of plants within a community is dictated to some extent by their association with microorganisms in the soil. That association is affected by root exudates and possibly by their quality. The competitiveness of species under various grazing regimes has been defined by their response to grazing as decreaser, increaser, or invader. To test the hypothesis that there are recognisable differences in the chemical fingerprints of the root exudates of decreasers, increasers and invaders, seeds of 8 grasses, representing these 3 designations, were germinated and grown for 2 weeks in a root exudate trapping system in the laboratory. Tentative identification of the suite of compounds recovered from the root exudates by a solvent extraction technique was done with the help of gas chromatography/mass spectrometry and authentic samples. Eleven identified compounds, present in all exudates as major peaks, but absent in the blanks, were selected for semi-quantitatively comparing the 3 grazing response groups. For all 11 compounds, there was always at least 1 of the grazing response groups that had the highest percentages. That is to say, they were qualitatively, based on the 11 compounds selected, but not quantitatively similar.

Resumen

La competitividad de las plantas dentro de una comunidad es dictada en parte por su asociación con los microorganismos del suelo. Esa asociación es afectada por los exudados de la raíz y posiblemente por su calidad. La competitividad de las especies bajo varios regímenes de apacentamiento ha sido definida por su respuesta al apacentamiento como especies decrecientes, crecientes o invasoras. Para probar la hipótesis de que hay diferencias reconocibles en la huella química de los exudados de la raíz de especies decrecientes, creciente e invasoras se tomaron semillas de 8 zacates que representan estas 3 designaciones y se geminaron y crecieron por dos semanas en un sistema de laboratorio que atrapaba los exudados de la raíz. La identificación tentativa del grupo de compuestos de los exudados de la raíz recuperados mediante una técnica de extracción con solventes se hizo con la ayuda de cromatografía de gases y espectrofotometría de masas y muestras auténticas. Once compuestos identificados, presentes en todos los exudados como picos principales, pero ausentes en los blancos, se seleccionaron para comparar semicuantitativamente los 3 grupos de respuesta al apacentamiento. Para todos los 11 compuestos, siempre hubo al menos 1 de los grupos de repuesta al apacentamiento que tuvo los mas altos porcentajes. Es decir, ellos fueron cualitativamente, basados en los 11 compuestos seleccionados, pero no cuantitativamente similares.

Key Words: organic acids, native grasses, introduced grasses, soil quality, soil chemical properties

Rangeland plant species have been classified as decreasers, increasers, or invaders (Bedell 1998). Decreaser species often dominate the undisturbed grassland communities and normally have large plants. Decreaser species derive their classification from their response to grazing pressure. Increaser species are those that replace the decreasers and are more resistant to grazing pressure while invader species are normally aliens that are capable of competing in a grazed or ungrazed environment.

The consequence of replacing the dominant species in a plant community is a seral stage that can be resistant to succession (Dormaar and Willms 1990, Dormaar et al. 1994). For example, blue grama (*Bouteloua gracilis* (HBK.) Lag. Ex Steud) appears to resist displacement by its associated climax dominant species. Similarly, crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), an introduced species, is a strong competitor in Mixed Prairie communities with the ability to invade and colonize undisturbed native communities (Caldwell 1991, Trent et al. 1993).

The competitiveness of plants within a community is dictated to some extent by their association with microorganisms in the soil such as the vesicular-arbuscular mycorrhizal type. That association is affected by root exudates and possibly by their chemical quality (Bokhari 1978, Klein et al. 1988, Foster and Dormaar 1991). Inhibitors of nitrifying bacteria were found in the root extracts of increaser and invader species (Neal 1969). Conversely, Bremner and McCarty (1993), based on an intensive literature survey, concluded that there was no satisfactory evidence to support the hypothesis that grasses inhibit nitrification in soils by exuding substances that retard oxidation of NH_4+ by nitrifying microorganisms. A later study by Neal (1973) showed that invader species had increased phosphatase activity, reflecting a potential for higher P uptake, in the surrounding soil as com-

The technical assistance of R. R. James with the initial stages of this research project and of J. Elder who conducted the GC-MS analyses is gratefully acknowledged.

Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warrantee of the product by Agriculture and Agri-Food Canada and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Lethbridge Research Centre Contribution No.387-0089
Manuscript accepted 17 Sept. 01.

pared to dominant, co-dominant, or increaser species.

Organic acids play a prominent role in cell metabolism, affect rhizosphere pH, and microbial activity (Curl and Truelove 1986). They are good metal-chelating compounds and aid in the absorption and translocation of nutrient elements. Rice (1984), after having summarised what could be found in the literature, concluded that organic acids are important in allelopathy. It was speculated that i) 3 major long-chain fatty acids, i.e., palmitic, stearic, and arachidic acids, identified from soil in rough fescue (*Festuca campestris* Rydb.) grasslands that were in good condition because of low grazing pressure, could well contribute to the resistance of those grasslands to the encroachment of invading species (Dormaar and Willms 1992) and ii) dioctyl adipate or di(2-ethylhexyl)phthalate could be linked to inhibition of nitrification (Dormaar et al. 1994). Nevertheless, in spite of these roles and speculations, organic acids are rarely included in lists of exudate components (Curl and Truelove 1986).

Before establishing if there is a possible relationship between exudate composition and the competitive status of plant species, fingerprint organic acid compositions, in the form of gas chromatographic patterns, of root exudates from the first 2 weeks of growth of a number of decreaser, increaser, and invader grass species will have to be obtained. This would then allow testing the hypothesis that there are recognisable semi-quantitative differences in the chemical fingerprints of root exudates from plants that fall within these designations.

Materials and Methods

Species Selected

Seeds of 5 native and 3 introduced, invading grass species were collected in the field: Needle-and-thread (*Stipa comata* Trin. and Ruper.), Western wheatgrass (*Agropyron smithii* Rydb.), and rough fescue were selected to represent decreaser species; blue grama and June grass (*Koeleria cristata* (L.) Pers.) were selected to represent increaser species; and crested wheatgrass, Russian wildrye (*Elymus junceus* Fisch.) and timothy (*Phleum pratense* L.) were selected to represent invader species. The seeds were collected by glove-covered hands. They were then placed in paper bags and stored at room temperature. To simulate field conditions, the seeds were not sterilised prior to use.

Collection of Root Exudates

Seeds were placed in a thin bed of finely (particle size 0.5 to 1.0 mm) crushed lava rock over a deeper bed of 2-cm size lava rock. Blanks were the same but without the seeds. The lava rock was obtained from a garden centre. After emergence, they were thinned down to aim for the same amount (100 if possible; if less they were counted) of seedlings and allowed to grow for 2 weeks after germination. The seeds and plants were irrigated with 50-ml 0.1-strength Hoagland solution. This was then circulated at the rate of 20-ml min^{-1} by air-lift. Water was added to compensate for losses due to aspiration and evaporation.

The exudates were trapped with a continuous hydrophobic root exudate trapping system (Tang and Young 1982). This technique was selected as a first approximation of either different compounds or the same compounds at different quantities. To eliminate equipment related contaminants, a smaller, all glass and Teflon™ system was designed. Prior to assemblage of the system, both the 2-cm size and finely crushed lava rock were heated to 900° C, while the all quartz glass wool was heated to 600° C. The quartz glass wool was placed at the bottom of a 725-ml funnel-shaped growth reservoir. Next, the 2-cm size lava rocks were added which in turn were covered with the finely crushed lava rock. The thistle of the funnel was attached, via a taper-ground joint, to a 19 x 150 mm glass tube. Root exudates were collected on a 6-cm resin bed of Bio Beads™ SM4 (20-50 mesh). Prior to use, the Bio Beads™ were Soxhlet-extracted (Tang and Young 1982) with acetone, acetonitrile, and diethyl ether each for 24 hours to eliminate any potential impurities in the product, and stored in glass-distilled methanol. All solvents were glass distilled OmniSolv™ grade. When in the collection cylinder, the Bio Beads™ were rinsed with 10-bed volumes of double, glass distilled water. The containers and collection cylinders were wrapped in aluminum foil. Duplicate runs were made of a control, without plants, with each set of 4-plant systems. Runs were continued until 2 consecutive ones of a plant species were obtained with good duplication. That is, considering all steps adding to errors and the accumulative errors of the calculations, replicate runs with about 3% variation were considered acceptable precision for this study.

Analysis

Following the 2-week growth period, the resin beds were rinsed with 5-bed volumes of glass distilled water to eliminate any water-soluble compounds. Next, the beads were extracted with 5-bed volumes of glass distilled methanol. The methanol extract was reduced on a rotary evaporator (60° C, 30 kPa). The remaining 2 ml was adjusted to pH 2.0 with 3N HCl. Ten microlitres of 1-mg ml^{-1} methanol stock of suberic acid (octanedioc or 1,6-hexanedicarboxylic acid) were added to serve as internal standard. This mixture was then extracted 3 times with 25-ml CH_2Cl_2, dried over anhydrous Na_2SO_4, and reduced to 1-ml with a stream of N_2 at room temperature. One half ml of the sample was esterified by adding 1-ml 14% boron trifluoride in methanol and placing it in a 60° C water bath for 10 min. Following cooling, 1-ml saturated sodium chloride in glass distilled water was added. After shaking, 1-ml hexane was added. After 10 min, 400µl of the hexane solution was placed in a vial for analysis.

Initial analyses (1µl) were carried out with a Hewlett Packard GC 5890 Series II Plus, using a 30-m long capillary column (i.d. 0.320 mm) wall-coated with 5% diphenyl-95% dimethyl polysiloxane (DB-5) with helium as the carrier gas (about 1 ml min^{-1}). The operating conditions were: 50° C for 1 min, then 25° C min^{-1} to 100° C for 1 min, then 3° C min^{-1} to 275° C for 1 min; the injection port temperature was 250° C, while the flame ionization detector temperature was 275° C. The qualitative analyses were carried out with a Hewlett Packard GC-MS data system. Following tentative identification, the spectra of the major peaks selected were compared with authentic samples.

Over 70 compounds, many in minute quantities, were tentatively identified. Of these, only 11, which were present in all exudates across the spectra but absent in the blanks, were selected for comparison. They were then calculated as percent against the standard (octanediocic acid) and adjusted for 100% germination of 100 seeds. Another way could have been to normalise the exudate composition by root weight. However, root washing, no matter how carefully done, leads to losses of root hairs and thus considered inappropriate. The presence of seed remnants and the ash content of the root mass made weighing the burned lava rock before and after inappropriate as well. In this study, precision of the chromatographic output was considered of greater value in fingerprint separation than accuracy.

A test of the identified compounds among the grazing response groups was evaluated as a simple ANOVA where each

Table 1. Tentatively identified methylated carboxylic acids (A) in root exudates collected over a 2-week period of growth following germination as determined by GL-MS.

	Indigenous					Non-indigenous Invaders		
	Decreaser			Increasers				
	Stipa comata	*Agropyron smithii*	*Festuca campestris*	*Bouteloua gracilis*	*Koeleria cristata*	*Agropyron cristatum*	*Elymus junceus*	*Phleum pratense*
Octanoic A	7[1]	15	8	36	30	12	17	22
Nonaoic A	27	29	30	93	82	116	99	115
1,2-Benzenedicarboxylic A[2]	29	25	27	14	10	9	12	19
Dodecanoic A	11	20	8	11	8	58	41	60
Nonanedioic A	8	15	6	11	5	27	28	29
Tridecanoic A	32	29	31	4	6	14	12	13
Teradecanoic A	10	10	12	93	87	27	35	29
Pentadecanoic A	3	9	10	42	40	11	16	9
2-HexadecanoicA (formII)	45	18	19	16	18	79	85	101
1,2-Benzenedicarboxylic A[2]	39	48	69	7	13	24	14	20
E-9-Octadecenoic A	9	14	6	24	33	99	113	106

[1]The numbers represent the percent against the standard (Octanedioic A) and are adjusted for 100% germination of 100 seeds.
[2]Dimethyl and butyl-ethyl, respectively.

species represented a replicate. Consequently, the design was unbalanced with 3 decreaser, 2 increaser, and 3 invader replicates. Treatment means were compared with single degree of freedom contrasts.

Results and Discussion

All 11 tentatively identified compounds (Table 1) revealed separation ($P < 0.01$) among the grazing response groups (Table 2) suggesting specific semi-quantitative fingerprint patterns. There was no consistent ranking in the magnitude of the organic compounds produced by each group. Nevertheless, for all 11 compounds, there was always at least 1 of the grazing response groups that had the highest percentages. In spite of all precautions, because of the polystyrene composition of the Bio Beads™, the blank runs still had 'noise.' It is, therefore, acknowledged that by concentrating on the 11 major peaks not present in the 'noise', the potential significance of minute quantities of specific compounds, present in some but possibly absent in other root exudates, but masked by the 'noise', could potentially give qualitative evidence for the decreaser/increaser/invader designation.

It is unfortunate that the level of 1,2-benzenedicarboxylic acid or phthalic acid must always be suspect when working with soil extracts. Due to the increased use of plastics over the last 50 years (Dormaar 1982), phthalic acid has more or less become a universal contaminant. However, under the conditions of the experiment the assumption is being made that the phthalic acid measured is a true product of the root exudates obtained and processed, since it was not present in the blanks.

Soil quality changes manifest themselves through changes in quantity and quality of the chemical changes of root exudates (Dormaar and Willms 1995). Although decreasers are plants that may well succumb to grazing pressure, increasers and invaders may partially invade space vacated by the decreasers, the chemical potential always exists that root exudates, albeit ephemeral and minor in terms of contribution to total soil C, may prevent decreasers from returning or plants in general from re-entering higher successional states. Callaway and Aschehoug (2000) found that diffuse knapweed's (*Centaurea diffusa* Lam.) advantage against North American species appeared to be due to differences in the effects of its root exudates and how these root exudates affected competition for resources. No specific compounds were identified.

Dormaar et al. (1980) and Dormaar (1982) demonstrated that gas chromatographic patterns of solvent extractable organic acids from Chernozemic soils could be related to the vegetation growing on those soils. However, this present study did not elucidate why some plants are decreasers, while others are increasers or invaders, even though they did have different semi-quantitative gas chromatographic fingerprint outputs. Without knowing what even the tentatively compounds do in the rhizosphere, no conclusions towards this can even be proposed. Neither may the identified compounds be necessarily in the soil as the same semi-quantitative fingerprint. For example, Biondini et al. (1988) established that microorganisms, in sterile and non-sterile fritted clay used as growth media, significantly increased root exudation of *A. cristatum* and *A. smithii*, but had no effect on *B. gracilis*. Their data suggested that an introduced plant species may be markedly different from native species in the shortgrass steppe in terms of exudate releases. No specific compounds were identified.

This study was designed, as a first step, to obtain a qualitative comparison of a group of compounds identified in root exudates collected over a 2-week period. Little, if any is known about the potential

Table 2. Statistical comparisons of tentatively identified methylated carboxylic acids (A) of root exudates among species groups (shown in Table 1).

	Treatment effect	Decreaser vs Increaser	Decreaser vs Invader	Increaser vs Invader
	----------Probability----------			
Octanoic A	0.008	0.003	0.122	0.012
Nonaoic A	<0.001	<0.001	<0.001	0.017
1,2-Benzenedicarboxylic A[1]	0.009	0.007	0.006	0.710
Dodecanoic A	0.002	0.642	0.002	0.002
Nonanedioic A	0.002	0.633	0.002	0.002
Tridecanoic A	<0.001	<0.001	<0.001	0.001
Teradecanoic A	<0.001	<0.001	0.001	<0.001
Pentadecanoic A	<0.001	<0.001	0.150	<0.002
2-HexadecanoicA (formII)	0.002	0.392	0.002	<0.001
1,2-Benzenedicarboxylic A[1]	0.012	0.007	0.012	0.372
E-9-Octadecenoic A	<0.001	0.017	<0.001	<0.001

[1]Dimethyl and Butyl-ethyl, respectively.

effect of the individual compounds identified. Although the bleeding of resins will have to be explored further, it is suggested that the next step ought to be to take the 11 compounds identified and to test their effect on some sensitive plants, such as lettuce or cucumber, as a first approximation. However, even if this leads to further specific insights of the compounds identified, in the final analysis it may well be a combination, and most likely synergistic at that, of a number of factors, such as resource competition (moisture, nutrients), aggressive growth behaviour, disease resistance, and chemical differences in root exudates, that enable plants to act as decreasers, increasers or invaders. In terms of the study under discussion it can be concluded, as Dormaar and Willms (1990) did with NaOH-soluble organic acids between soils of needle-and-thread and blue grama range, and cropland, that the suite of constituents selected and identified were qualitatively, but not semi-quantitatively similar.

Literature Cited

Bedell, T.E. 1998. Glossary of terms used in range management, 4th edition. Glossary Update Task Group, Soc. Range Manage., Denver, Colo.

Biondini, M., D.A. Klein, and E.F. Redente. 1988. Carbon and nitrogen losses through root exudation by *Agropyron cristatum, A. smithii* and *Bouteloua gracilis*. Soil Biol. Biochem. 20: 477–482.

Bokhari, U.G. 1978. Allelopathy among prairie grasses and its possible ecological significance. Ann. Bot. 42: 127–136.

Bremner, J.M. and G.W. McCarty. 1993. Inhibition of nitrification in soil by allelochemicals derived from plants and plant residues. *In*: J.-M. Bollag and G. Stozky (eds.), Soil Biochemistry 8: 181–218. Marcel Dekker Inc., New York, N.Y.

Caldwell, M. 1991. Underground events may be root of grass behavior. Utah Science 53 (2): 51.

Callaway, R.M., and E.T. Aschehoug. 2000. Invasive plants versus their new and old neighbours: a mechanism for exotic invasion. Sci. 290: 521–523.

Curl, E.A. and B. Truelove. 1986. The rhizosphere. Springer-Verlag, Berlin, Germany.

Dormaar, J.F. 1982. Aliphatic carboxylic acids in Chernozemic soils. Can. J. Soil Sci. 62:487–494.

Dormaar, J.F. and W.D. Willms. 1990. Effect of grazing and cultivation on some chemical properties of soils in the Mixed Prairie. J. Range Manage. 43:456–460.

Dormaar, J.F., and W.D. Willms. 1992. Water-extractable organic matter from plant litter and soil of rough fescue grassland. J. Range Manage. 45:152–158.

Dormaar, J.F. and W.D. Willms. 1995. Root mass and quality as a measure of range health, p.121. *In:* Proc. 5th Int. Rangeland Congress, Volume 1, Salt Lake City, Ut.

Dormaar, J.F., B.W. Adams, and W.D. Willms. 1994. Effect of grazing and abandoned cultivation on a *Stipa-Bouteloua* community. J. Range Manage. 47:28–32.

Dormaar, J.F., A. Johnston, and S. Smoliak. 1980. Organic solvent-soluble organic matter from soils underlying native range and crested wheatgrass in southeastern Alberta, Canada. J. Range Manage, 33:99–101.

Foster, R.C. and J.F. Dormaar. 1991. Bacteria-grazing amoebae in situ in the rhizosphere. Biol. Fertil. Soils 11: 83–87.

Klein, D.A., B.A. Frederick, M. Biondini, and M.J. Trlica. 1988. Rhizosphere microorganism effects on soluble amino acids, sugars and organic acids in the root zone of *Agropyron cristatum, A. smithii* and *Bouteloua gracilis*. Plant Soil 110:19–25.

Neal, Jr., J.L. 1969. Inhibition of nitrifying bacteria by grass and forb root extracts. Can. J. Microbiol. 15:633–635.

Neal, Jr., J.L. 1973. Influence of selected grasses and forbs on soil phosphatase activity. Can. J. Soil Sci. 53:119–121.

Rice, E.L. 1984. Allelopathy, 2nd ed. Academic Press, Inc., Orlando, Fla.

Tang, C.-S. and C.C. Young. 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bigalta limpograss (*Hemarthria altissima*). Plant Physiol. 69: 155-160.

Trent, J.D., A.J. Svejcar, and G.J. Bethlenfalvay. 1993. Growth and nutrition of combinations of native and introduced plants and mycorrhizal fungi in a semiarid range. Agr., Ecosystems, Environ. 45: 13-23.