In situ extraction of rhizosphere carbon from native and invasive plant species

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In Situ Extraction of Rhizosphere Carbon from Native and Invasive Plant Species

by

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B.S. Cornell University, 1992

presented in partial fulfillment of the requirements
for the degree of
Master of Science
The University of Montana
1998

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Date
5-14-98
In Situ Extraction of Rhizosphere Carbon from Native and Invasive Plant Species

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The rhizosphere environment is an integral part of the plant-soil continuum. Defined as the narrow zone of soil that is under direct influence of plant roots, the rhizosphere is an area of intense biological activity compared to the bulk soil. Rhizodeposition products released from plant roots (exudates, secretions, lysates and gases) affect soil processes and soil microbial communities within the rhizosphere. These soil processes and microbial communities, in turn, influence plant growth and community structure. Despite the importance of the interaction between the plant, soil, and soil microbial populations resulting from rhizodeposition products, there is currently no adequate means of capturing, identifying and quantifying these products in situ. This research explored the use of non-ionic carbonaceous resins for capture, identification and quantification of rhizodeposition products in situ. First, this research addressed the comparison of three different resin types and their ability to adsorb and desorb three compounds of varying chemical complexity that are similar to compounds found in the rhizosphere soil. Different extraction sequences and extraction mechanisms were also tested for their ability to remove sorbed compounds from the resins. The second part of this research investigated the use of non-ionic carbonaceous resins for in situ capture, identification and quantification of rhizosphere C from the rooting zones of spotted knapweed and Idaho fescue. Specifically, differences in hexose sugars (ARC) and total organic carbon (TOC) between spotted knapweed, Idaho fescue and bulk soil were identified. Ambersorb 563 resin, dropwise extraction mechanism and a sequential extraction using water, 50% and 100% methanol were used. The Amberlite XAD-7 resin may prove superior for capture and release of polyphenolic compounds. In rhizosphere studies, knapweed consistently had greater sugar and TOC concentration in the rhizosphere than Idaho fescue or bulk soil.
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Acknowledgements

I would first like to thank my parents for their never ending support and love and their ability to give me a good kick in the butt when I need it. I also want to thank my sister, my best friend in the world, for always being there and for giving me that bloody nose in 3rd grade which brought me around and set me on the right track. I am also grateful to my advisor Tom DeLuca for his notable ability to acquire funding, his advice and encouragement and his unbelievable patience when I faced eight months of writers’ block. Also deserving attention are my committee members Cathy Zabinski and Don Bedunah for their sound advice and support. This research would not have been possible without the help of Geoff Richards, Bev Parker and Glenn Ponder in the Carbohydrate Chemistry Lab and Jim Gannon in Microbiology. Their generosity and technical support was an integral part of the success of this project. Special thanks to Todd Wojtowicz for help in the field and for his caustic conversation which made life just a little more interesting. Thanks to all the people in the soils group for their friendship and support: Vince Archer (just for listening), Ula Choromanska (gin and tonics and vodka), Julie Knudsen (hiking and biking), Jenny Newland (Star Wars trilogy), Jay Norton (phosphorus financial support), Mark VanderMeer (R & R - pool at Trails End and trail work) and Kris Zouhar (resin capsule extraction). And finally, thanks to George Mathieus (HUNTIN’-FISHIN’!!) for his friendship and support while this thesis was being written.
Preface

Rhizodeposition products released from plant roots affect soil processes and soil microbial communities within the rhizosphere, which, in turn, affect plant growth and development. There is currently no adequate means of capturing, identifying and quantifying rhizodeposition products in situ with little disturbance to the plant and the soil environment. The work reported herein investigated the use of non-ionic carbonaceous resins for the adsorption, release and quantification of rhizodeposition products from the rooting zone of *Centaurea maculosa* and *Festuca idahoensis*.

The first chapter describes development of the methodology for using non-ionic carbonaceous resins to capture organic compounds. Information was needed regarding the adsorption/desorption abilities of a three resins as well as the best methods and extracting agents for compounds sorbed to the resins. Three compounds (similar to those compounds which may be found in rhizosphere soil) of varying chemical complexity were used to test the adsorption/desorption ability of resins.

Since adsorbed compounds must be released from the resins in order to be identified and quantified, two extraction solvent sequences using K$_2$SO$_4$ or water followed by a final 90% methanol:water rinse were tested for effectiveness in removing glucose sorbed to a resin. The most effective and time efficient way to remove compounds also needed to be determined; therefore a variety of extraction mechanisms were tested.

The second chapter investigates the use of non-ionic carbonaceous resins for in situ capture, identification and quantification of rhizosphere C from the rooting zones of...
spotted knapweed and Idaho fescue. Specifically, differences in hexose sugars (ARC) and total organic carbon (TOC) between spotted knapweed, Idaho fescue and bulk soil were identified.
Chapter 1.

Comparison of Resins, Elution Techniques and Eluents for Adsorption and Desorption of Rhizosphere Compounds

ABSTRACT

Two non-ionic carbonaceous resins, Ambersorb 563 and Amberlite XAD-7, and an anion exchange resin, Amberlite IRA-68, were evaluated for their ability to adsorb and release glucose (a simple sugar), catechin (a flavonoid), and rutin (a glycosilated flavonoid). These compounds are representative of some of the compounds typically found in rhizosphere soil. Resins were placed in 100 ppm solutions of these three compounds and were eluted using a sequential extraction of 10 ml de-ionized water, 10 ml 50% methanol and 10 ml 100% methanol. Eluents were analyzed for glucose using anthrone method and for phenol content using the Folin-Denis method. Ambersorb 563 exhibited the best adsorption capacity for all three materials tested, however it did not have the best desorbing properties with the eluents used. Amberlite XAD-7 showed similar adsorption capacity compared to Ambersorb 563 across all compounds except for rutin and it allowed for the most effective desorption of the compounds tested. Elution of glucose from Ambersorb 563 was also tested using 2 extraction solvent sequences (H₂O successive wash and K₂SO₄ successive wash) and with 3 extraction mechanisms (drop-wise, sequential rinse and resin removal and agitation). Although these methods yielded similar results, the drop-wise extraction method was determined to be the most convenient means of glucose elution from the resin.
INTRODUCTION

Rhizodeposition products play an important role in many plant and soil microbial processes (Lynch and Whipps, 1991; Tate, 1995). A better understanding of these belowground processes is greatly enhanced by scientists' ability to capture and identify rhizodeposition products and their metabolites in-situ, an ability which is currently limited by inadequate methodology. Many studies use techniques in which the rhizosphere soil is separated from the bulk soil -- a process which is highly subjective and variable (Bhokari et al., 1975; Ortas, 1997) This method also requires destructive sampling of plant and soil, making it of little use in long-term studies investigating changes in relative amounts and composition of rhizodeposition products.

Techniques using $^{14}$CO$_2$ have proven useful for measuring C fluxes from the plant to the soil and microbial components of the rhizosphere. Two approaches are widely used for radio-carbon labeling of rhizosphere C: the pulse-chase method and continuous exposure of plant shoots to $^{14}$CO$_2$. In both techniques the shoots are separated from the root environment and exposed to $^{14}$CO$_2$. The labeled C is followed to determine where plant assimilated C ends up in the plant-soil continuum. The pulse-chase method, where shoots are exposed to labeled CO$_2$ for a brief period, tends to result in uneven labeling of C pools in the plant and provides information on recently fixed C. Plant structural components of rhizosphere C such as dead roots are largely ignored. Constant feeding techniques, where plants are exposed to a steady supply of $^{14}$CO$_2$, are more successful in giving an accurate representation of C movement and loss because of more homogenous labeling, but do not distinguish between root exudates and root turn-over. Both
approaches also require a highly controlled growing environment and destructive sampling which prevents long-term studies investigating changes in composition and quantity of rhizodeposition through time. Although radio-C labeling techniques are useful in providing certain information about plant root exudates, another method is needed for capture and identification of rhizodeposition products in situ.

The success of ion exchange resin (IER) bags in extracting plant nutrients (particularly mineral N in the form of NH$_4$ and NO$_3$) from the soil is well documented (Binkley et al 1986, Binkley and Hart 1989). A known volume of anionic and cationic resins is placed into mesh bags and incubated in the ground for a specified amount of time. After the bags are removed the resins are extracted with a salt solution to determine the amount of ions adsorbed and nutrient availability is determined. Despite the success of IER technology in determining soil fertility, little work has focused on the use of non-ionic carbonaceous resins (NICR) in plant and soil sciences. Traditionally, these resins are used in removal of organic pollutants from wastewater, groundwater and vapor streams. They have a chemical structure similar to graphite, containing many C-C bonds with few functional groups and have sorption characteristics similar to activated C (Johns and Skogley, 1994). Recent research has shown that NICR's can act as non-specific organic sinks able to extract sugars from soil pastes and soil solution (Johns and Skogley, 1994).

Preliminary research by DeLuca (1995, unpublished) and Morse (Chapter 2) using NICR has shown that these resins may be useful in capturing and identifying root exudates in situ from plant rhizospheres. Capsules containing Ambersorb 563 NICR were placed into the rooting zone of spotted knapweed and Idaho fescue plants, extracted
and analyzed for sugars, amino N and phenols. Analysis of the extracted capsules indicated that capsules placed in the rhizosphere of spotted knapweed contained 2 to 10 times more hexose sugars when compared to capsules placed in the rhizosphere of Idaho fescue. This was further supported by higher total C levels observed in knapweed extracts (Chapter 2). More information is needed regarding sorption/de-sorption capabilities of various NIRC’s as well as the best methods and extracting agents for elution of compounds sorbed to the resins.

PURPOSE AND OBJECTIVES

The purpose of this set of studies was to compare the ability of two NICR’s and one IER to adsorb and to have de-sorbed, through sequential extractions, three types of organic compounds similar to those found in plant rhizospheres. Three different elution methods and 2 extraction compounds were also tested for their efficacy removing resin sorbed compounds. The specific objectives of these studies were to:

1) Compare the ability of Ambersorb 563, Amberlite XAD-7, and Amberlite IRA-68 to adsorb and release glucose, catechin and rutin.

2) Compare the ability of 2 extraction solvent sequences: H₂O successive wash and K₂SO₄ successive wash to remove glucose adsorbed to Ambersorb 563, Amberlite XAD-7 and Amberlite IRA-68.

3) Compare 3 extraction mechanisms: drop-wise, sequential rinse, and resin removal and agitation, to release glucose adsorbed to Ambersorb 563.
MATERIALS AND METHODS

Resins

The non-ionic carbonaceous resins used in the studies were Ambersorb 563 and Amberlite XAD-7. The ion-exchange resin used was Amberlite IRA-68, a weakly basic anion exchange resin. All Amberlite and Ambersorb resins are a trademark of Rohm and Haas Co., Philadelphia, PA. These resins were selected because of their non-specificity and wide range of adsorption capabilities. Such traits are necessary to allow the sorption of common root exudate signals which may be hydrophobic or hydrophilic in nature or of low or high molecular weight.

Resin Elution Technique

Several means of glucose elution from Ambersorb 563 were tested using 2 extraction solvent sequences (H₂O successive wash and K₂SO₄ successive wash) and 3 extraction mechanisms (drop-wise, sequential rinse and resin removal and agitation).

**Extraction solvent sequence:** The H₂O and K₂SO₄ successive washes were used to determine which eluent would be the most effective at removing glucose sorbed to NICR’s. Approximately 1.5 cm moist volume of each resin type was placed in the bottom of 1.5 cm diameter by 15 cm length of open-ended glass column. Fiberglass mesh screen overlaid with a layer of glass wool prevented the resin from falling through the opening in the bottom of the column but allowed for liquid to pass freely. An 8 cm length of plastic Tygon tubing was attached to the bottom of the glass column and sealed with a screw clamp.
Resins and apparatus were washed thoroughly with two 15 ml sequential rinses of 100 percent re-distilled methanol followed by four 15 ml sequential rinses of de-ionized water. Excess rinse water was forced out of the system and 10 ml of 25 ppm glucose solution was placed in the columns with the resin and stirred briefly with a glass rod to ensure mixing of resin and solution. The solution remained in the column with the resin for an hour at which time the solution was drained from the column into a sample bottle which was sealed and refrigerated for future chemical analysis.

The resins in the column were eluted by 4 successive 10 ml washes of either de-ionized water or 0.4 M $K_2SO_4$. The fifth and final wash for each eluent type was 10 ml of 90% methanol. Each of the successive washes was collected for chemical analysis.

**Extraction mechanisms:** The 3 extraction mechanisms (drop-wise, sequential rinse and resin removal and agitation) were tested for which was most effective at recovering glucose sorbed to Ambersorb 563 resin. Polyester mesh capsules containing 1100 m² surface area of Ambersorb 563 resin were allowed to soak in a 25 ppm dextrose solution for 1 hour. The solution was decanted and the capsules were extracted by one of three methods.

1) **Drop-wise:** Capsules were eluted by dripping eluent at a rate of 1 drop/sec as 25 ml each of d.i. water, 50 % methanol and 100 % methanol through the capsule. Half way through each successive rinse (12.5 ml) the capsule was vertically rotated to ensure complete elution of the capsule from both sides. Each of the successive rinses was collected for chemical analysis.

2) **Sequential rinse:** Capsules were eluted by placing an intact capsule into 25 ml each of d.i. water, 50 % methanol and 100 % methanol. The capsules remained in each
sequential rinse for ten minutes. Each of the successive rinses was collected for chemical analysis.

3) Removal and agitation: Capsules were eluted by removing resin beads from the capsules and placing in 25 ml each of d.i. water, 50 % methanol and 100 % methanol and placed on a shaker at low speed. Resin remained in each sequential rinse for ten minutes. Each of the successive rinses was collected for chemical analysis.

**Sugar and Polyphenol Adsorption/Desorption Efficiency of Resins**

The apparatus and methodology for the following set of studies is similar to that which was described for the extraction solvent sequence study (see above).

Dextrose (D-(+)-glucose, Figure 1a) was used for sugar adsorption. Catechin ((+)-catechin hydrate, Figure 1b) and rutin hydrate (Figure 1c) were used for flavonoid and glycosilated flavonoid adsorption, respectively. Each of these compounds was used to make 100 ppm aqueous solution in 100 ml volumetric flasks. The rutin was first dissolved in 20 ml of 100 percent methanol and then brought up to volume with de-ionized water.

The solutions remained in the columns with the resin for a half hour at which time the solution was drained from the column into a sample bottle which was sealed and refrigerated for future chemical analysis. The resin in the column then underwent three sequential rinses of: 1) 10 ml de-ionized water, 2) 10 ml 50 percent re-distilled methanol/H$_2$O solution, and 3) 10 ml 100 percent methanol solution. Each of these rinses was collected, sealed, and refrigerated for chemical analysis.
Chemical Analysis of Samples

Glucose and glycoside analyses were determined using the anthrone reagent (DeLuca, 1998). Polyphenol analyses (catechin and rutin) were performed using the Folin - Denis method for phenol determination (Stern et al., 1996).

Statistical Analysis

One-way ANOVA tests were used to determine differences in the ability of the resins to absorb and desorb the compounds and also to determine effectiveness of each sequential rinse to desorb the compounds. Tukey’s HSD was used to determine mean separation with an alpha level of 0.05.

RESULTS AND DISCUSSION

Resin Elution Technique

Extraction Solvent Sequences: There were no significant differences at alpha level 0.05 between the H₂O and K₂SO₄ successive washes (F=3.20, p=0.08). Both eluents exhibited the ability to recover, on average, anywhere between 20 to 35 percent of the glucose that was adsorbed to the resin. Most of the glucose was recovered in the first rinse with the amount decreasing with each sequential rinse. The amount of glucose recovered in the first rinse was significantly higher than that recovered in each of the following rinses (F=25.18, p=0.0001)

Although not tested explicitly, the most effective solvent sequence, based on this study and results from field studies presented in the following chapter, was determined to be a water, 50% methanol and 100 % methanol extraction. It is a simple, relatively
inexpensive way to recover a wide range of compounds, both hydrophilic and hydrophobic, that are sorbed to the resins. Fourth order eluents such as hexane or chloroform could be added as well if less polar compounds are to be eluted from the resins.

**Extraction Mechanisms:** There were no significant differences at alpha level 0.05 between the 3 extraction methods tested in this set of studies ($F=3.36$, $p=0.09$). The resin removal and agitation method exhibited the greatest tendency to recover sorbed glucose, followed closely by the drop-wise method. Due to the ease at which the drop-wise method can be carried out compared to the resin removal and agitation method, it was determined that drop-wise would be equally effective and the least time-consuming method.

**Glucose Adsorption and Desorption Study**

Ambersorb 563 exhibited the best adsorption capacity with 54% of the glucose in solution being adsorbed to the resin. Amberlite XAD-7 was significantly different ($F=9.89$, $p=0.005$) from the Ambersorb 563 showing the weakest affinity for glucose with only 40% of the glucose in solution adsorbed (Figure 2).

Glucose was most readily desorbed from the Amberlite XAD-7 resin. Approximately 75% of the glucose that was adsorbed to XAD-7 was removed by the three sequential rinses. This was significantly different ($F=4.63$, $p=0.02$) from Amberlite IRA-68 from which the smallest amount of glucose, approximately 58%, was desorbed. Sixty-five percent of the glucose that was adsorbed to Ambersorb 563 was desorbed in the rinses.
As would be expected, the water rinse removed most of the adsorbed glucose, as glucose is a hydrophilic compound. Approximately 51% of the adsorbed glucose was eluted by water across all three resins. The 50% methanol rinse desorbed 11% while the 100% methanol rinse desorbed 5%.

**Catechin Adsorption and Desorption Study**

The resins exhibited similar adsorption tendencies for catechin. All three resins adsorbed between 82 and 90 percent of catechin from the original solution with no significant differences between resins (Figure 2).

Catechin was most readily desorbed by the sequential rinses from Amberlite XAD-7. On average, approximately 30% of the catechin that was adsorbed to XAD-7 was removed in the sequential rinses. This was significantly higher ($F=145.31, p=0.001$) than the Ambersorb 563 and Amberlite IRA-68 showed decreased desorption, approximately 5% of the original amount adsorbed, when compared to XAD-7 (Figure 4).

The 100% methanol solution appeared to be the most effective desorption agent across all resins compared to the 50% methanol and water rinses. On average, approximately 23% of the catechin was desorbed with the 100% methanol rinse across all resins.

**Rutin Adsorption and Desorption Study**

Ambersorb 563 proved the most effective at adsorbing the rutin from solution by sorbing approximately 92%. Amberlite XAD-7 and IRA-68 were significantly lower
(F=131.40, p=0.0001) than the Ambersorb 563, adsorbing approximately 65% and 62%, respectively (Figure 2).

Rutin was most effectively desorbed from the Amberlite XAD-7 resin, with approximately 30% of the adsorbed material coming off in the sequential rinses. This was significantly different (F=49.92, p=0.0001) from the other two resins where only 13% of the rutin was desorbed from the Ambersorb 563 and 5% from the Amberlite IRA-68 (Figure 5). Similar to the catechin sorption study, most of the adsorbed material was removed by the 100% methanol rinse (Figure 5).

**CONCLUSIONS**

Due to the wide variety of compounds that are found in the rhizosphere soil finding a resin that effectively adsorbs and then releases 100% of these materials is perhaps unrealistic. What is needed is a broad spectrum resin that adsorbs most materials effectively and allows them to readily be desorbed by a certain eluent sequence. This will give researchers a firm idea of the types and relative quantities of compounds found as rhizodeposition products.

The NICR Ambersorb 563 appears to be the resin that most effectively adsorbs all three types of organic materials tested. However, it does not exhibit the best desorption capabilities. The materials tested in this study are most easily desorbed from the Amberlite XAD-7 followed by the Ambersorb 563 and then the IRA-68. A less polar solvent used as a desorbing agent (other than methanol or water), for example hexane or chloroform, may be more effective at removing the materials tested from the Ambersorb 563.
FIG. 1. Chemical structure of (a) dextrose; (b) catechin; (c) rutin used in the resin adsorption/desorption studies.
FIG 2. Percent glucose, catechin and rutin adsorbed to Ambersorb 563, Amberlite XAD7 and Amberlite IRA 68 resins.

FIG 3. Percent glucose desorbed in water, 50% and 100% methanol sequential rinses from Ambersorb 563, Amberlite XAD7 and Amberlite IRA 68 resins.
FIG 4. Percent catechin desorbed in water, 50% and 100% methanol sequential rinses from Ambersorb 563, Amberlite XAD7 and Amberlite IRA 68 resins.

FIG 5. Percent rutin desorbed in water, 50% and 100% methanol sequential rinses from Ambersorb 563, Amberlite XAD7 and Amberlite IRA 68 resins.
LITERATURE CITED


Chapter II

In Situ Extraction of Rhizosphere Carbon from Native and Invasive Plant Species

ABSTRACT

Currently there is no effective method for capture, identification and quantification of rhizodeposition products in situ. Polyester capsules (Unibest, Inc., Bozeman, MT) containing Ambersorb 563 (Rohm and Haas, Inc.) non-ionic carbonaceous resin were used to elucidate relative differences in the amount of rhizosphere carbon found in the rooting zone of spotted knapweed (Centaurea maculosa) and Idaho fescue (Festuca idahoensis). Plants were grown in greenhouse and field plot studies for approximately six weeks and 14 weeks, respectively. Resin capsules were inserted into the rooting zone of each plant species as well as a no-plant soil control and then analyzed for sorbed hexose sugars as anthrone reactive carbon (ARC), total organic carbon (TOC), and total phenols. Ultra-violet (UV) light spectrophotometric analysis and high performance liquid chromatography (HPLC) were also performed on resin capsule extracts. Capsules were extracted using a three-step sequential rinse: 25 ml de-ionized water, 25 ml 50 % methanol, and 25 ml 100% methanol. Greenhouse and field trials consistently showed 2 times more soluble C and 3-7 times more total soluble sugars in the rhizosphere of knapweed compared to Idaho fescue. Compounds sorbed to the resins from the knapweed rhizosphere were more effectively eluted by methanol than water and demonstrated both the presence of carbohydrate groups and UV absorption. This
indicates that sugars found in the rhizosphere of spotted knapweed may be glycosides associated with polyphenolic compounds. The UV spectrophotometric and HPLC analysis indicated that there are differences in the types of compounds found in the rhizosphere of spotted knapweed compared to that of Idaho fescue. Rhizosphere glycosilated polyphenolic compounds (e.g. flavonoids) may be released in greater mass by knapweed roots than fescue roots thereby influencing belowground ecology.

INTRODUCTION

Rhizosphere chemistry plays an important role in many plant and soil microbial processes. The rhizosphere is a zone rich in carbon (C) and nutrients and therefore is a zone of high biological activity. Much of the plant-derived C in the rhizosphere is exuded or secreted by active plant roots or is released from the decomposition of sloughed off root cells. Some studies have found up to 40% of the carbon fixed by plants is lost to the rhizosphere as rhizodeposition products, indicating that root exudation serves a definite physiological function (Lynch and Whipps, 1991).

Rhizodeposition products include amino acids, organic acids, pentose and hexose sugars, pyrimidines and pyridines, and enzymes which provide a source of readily available carbon and nutrients for soil microbial communities (Tate, 1995). This source of energy results in greater activity of microbes in rhizosphere soil compared to non-rhizosphere soil. Low-molecular-weight C compounds released into the soil as plant root exudates are readily available for microbial use and thus directly influence soil N and P transformations (Griffiths and Robinson, 1992; Tate, 1995). Increased concentration and activity in the soil microbial community results in higher decomposition rates of decaying
plant materials in the soil which then results in increased mineralization rates of organic N, P, and S into plant available forms (Tate, 1995). Rhizodeposition of these C compounds (specifically, organic acids, and phenols) also increases mobilization of soluble minerals such as Fe and Zn and root exudation is actually enhanced under mineral nutrient deficiency (Ratnayake et al., 1978; Marschner, 1996).

Plant growth and development are partly controlled by the soil environment of the rhizosphere; an environment the plant helps to create. Although it is well established that rhizodeposition products influence rhizosphere processes, it is not yet clear whether some plants are able to increase their competitiveness by altering the soil environment and the soil microbial communities adjacent to their roots.

Disruption of native plant community structure and function by exotic plant species is a problem threatening many of our natural and managed resources (Goodwin, 1991). Inadvertent and intentional transportation of alien plant species to North America, accompanied by the introduction of intensive grazing of livestock and fire suppression, has radically altered the health and appearance of our rangelands. In Montana and in most of the northwestern United States, spotted knapweed (Centaurea maculosa) is a noxious weed that is rapidly invading valuable range and forest land (Kelsey and Bedunah, 1989). Spotted knapweed is effective at colonizing disturbed sites and essentially establishing a monoculture, greatly decreasing the biodiversity of the original site, as well as greatly reducing the palatability and therefore the value of the range (Mooers and Willard, 1989). Once established, it may infest neighboring habitats that are relatively undisturbed.
Native range species such as Idaho fescue (*Festuca idahoensis*) may not be able to effectively compete with knapweed, and community structures are being radically altered. Effective control of invasive weed species is dependent on our understanding of the competitive advantages these alien weeds exhibit over native plant species.

Invasive plant species such as spotted knapweed may enhance their competitive abilities through alteration of the soil chemistry associated with their root system (Goodwin, 1992). Little is known about the rhizosphere dynamics of spotted knapweed and native graminoids such as Idaho fescue. To date, the science of root exudation and rhizosphere chemistry has been limited by a lack of methodology allowing soil and plant scientists to work with rhizosphere exudates *in situ*.

Present methodology for capture, identification and quantification of rhizodeposition products focuses primarily on highly artificial systems in which plants are grown in sterile nutrient solution culture or sand culture, neither resembling the complex soil environment in which plants are normally found (Lynch and Whipps, 1991). Although useful, these axenic systems fail to address important interactions between plants and associated soil microbial communities. Competition with other plant roots, nutrient limitations, water stress, temperature stress and microbial interactions, all important components of plant/soil interactions, are absent. Some studies have demonstrated that sterile rooting media may actually decrease root exudation as a result of the exclusion of microorganisms (Ratnayake et al., 1978; Marschner, 1996). Microbial communities are known to have a stimulatory effect on exudate production resulting from either: 1) microbial synthesis of hormones that stimulate plant growth and exudate production or 2) a feed-back mechanism in which microbial consumption of the exudates...
produces a chemical gradient through which exudates diffuse from the plant root to the soil (Tate, 1995).

Many studies investigating rhizodeposition in non-sterile soils use a technique in which the rhizosphere soil is separated from the bulk soil, a process which is highly subjective and variable (Bhokari et al. 1975; Ortas, 1994). This method requires destructive sampling of the plant and soil, making it of little use in long-term studies investigating changes in relative amounts and composition of rhizodeposition products through time.

Root dipping is another technique used for collecting root exudates from plants grown in non-sterile soil. With this method, plants are removed from soil, the roots are rinsed briefly and then are placed in a solution of 0.5 μM CaCl₂ where they remain for a specified amount of time. The solution is then analyzed for exudate compounds (Graham et al., 1981). This method also requires destructive sampling of the plant and may not give an accurate representation of which compounds are actually released into the soil.

Radio isotopes (¹⁴CO₂) have proven useful for measuring rhizodeposition rates in non-sterile soil, although there are problems associated with these techniques as well. The pulse-chase method, based on ¹⁴C found in rooting zone or as ¹⁴CO₂ in soil, tends to result in uneven labeling of C pools in the plant and therefore provides information on recently fixed carbon only. Constant feeding techniques are more successful in giving an accurate representation of C movement and loss, but also present the problem of destructive sampling and a highly controlled growing environment (Lynch and Whipps 1991). Although these methods are useful in providing certain information about plant
root exudates, another method is needed for capture, identification and quantification of rhizodeposition products in situ.

The success of ion exchange resin (IER) capsules in extracting plant nutrients from the soil is well documented (Binkley and Matson 1983, Binkley and Hart 1989). Despite the success of IER technology in determining soil fertility, little work has investigated the use of non-ionic carbonaceous resins (NICR) in plant and soil sciences. Traditionally, these resins are used in removal of organic pollutants from wastewater, groundwater and vapor streams. They have a chemical structure similar to graphite, containing many C-C bonds with few functional groups and have sorption characteristics similar to activated C (Johns and Skogley, 1993). Recent research has shown that NICR's can act as non-specific organic sinks able to extract sugars from soil pastes and soil solution (Johns and Skogley, 1994). Preliminary research by DeLuca (1995, unpublished) using a NICR has shown that these resins may be useful in capturing and identifying root exudates in situ from plant rhizospheres. Capsules containing Ambersorb 563 NICR were placed into the rooting zone of spotted knapweed and Idaho fescue and analysis of the extracted capsules indicated that capsules placed in the rhizosphere of spotted knapweed contained 2 to 10 times more hexose sugars when compared to capsules placed in the rhizosphere of Idaho fescue.

PURPOSE AND OBJECTIVES

The purpose of this study is to further investigate the use of non-ionic carbonaceous resins (NICR) for in situ capture, identification and quantification of rhizosphere C from the rooting zones of spotted knapweed and Idaho fescue. The specific objectives of the proposed study are to:
1) Investigate the efficacy of Ambersorb 563 NICR capsules for sorbing rhizosphere C in contrasting plant communities

2) Investigate differences in the quantity and composition of rhizosphere C between spotted knapweed, Idaho fescue and bulk soil.

MATERIALS AND METHODS

Greenhouse Experiment:

Spotted knapweed and Idaho fescue seeds collected from a local source were sown into flats containing potting soil. Eight weeks after germination, seedlings were transplanted into 4 inch diameter pots containing 400g soil. Soil was collected from a construction site on the southern end of Mt. Sentinel, Missoula, MT, air-dried and passed through a 0.5-cm sieve to remove larger stone fragments. The soil used in all studies was a fine, silty, mixed mesic Xeric Haploboroll with an average pH of 5.7 and a particle distribution of 29.8% sand, 26% silt, and 44.2% clay. At the time of transplant 3-cm diameter access tubes were inserted into the rooting zone of each pot to reduce root breakage and therefore to reduce increased exudation when resin capsules were inserted.

Plants were grown under natural light conditions and watered as needed for four weeks. At this time it was determined that sufficient root mass had developed for resin capsule insertion. Spotted knapweed was still in the rosette stage and the Idaho fescue was at the 4-5 tiller stage. Pots were rotated randomly on the greenhouse benches every week to help alleviate variation in light and temperature. Access tubes were removed and 2 cm diameter polyester mesh capsules containing 1100 m² surface area of Ambersorb 563 resin (Chapter 1) were inserted into the rooting zone of each pot. Before insertion,
capsules were washed thoroughly using a 100% methanol solution and 5 successive rinses of de-ionized water. Capsules were de-gassed for 24 hours following washing to remove air in pore spaces. After placement in the pots, resin capsules were wetted with 10 ml of de-ionized water and covered with soil. Pots were well watered after resin capsule placement.

Capsules remained in place for 10 days after which they were removed, thoroughly cleaned with a brush to remove soil adhered to the mesh and extracted using a constant drop rate from a buret set to deliver a three-step sequential rinse: 25 ml de-ionized water, 25 ml 50% methanol, and 25 ml 100% methanol (Chapter 1). Each fraction was filtered through a 1.2μm glass fiber filter paper and stored at -10°C until analyzed. Plants were harvested and shoots and roots were dried for 48 hours at 100°C. Soil samples were removed from each pot and soil moisture, ARC analysis and amino nitrogen analysis was determined on each sample (DeLuca, 1998). Soil samples of 25g were placed in 50 ml of 0.4 M K₂SO₄, shaken for 30 minutes and then filtered through 1.2 μm glass fiber filter paper.

Water, 50% methanol, 100% methanol and K₂SO₄ extracts were all analyzed for soluble hexose sugars using the anthrone reactive method (DeLuca, 1998) and analyzed for total phenolics using the Prussian blue method (Price and Butler, 1977). Total soluble C was analyzed in the water and K₂SO₄ extracts using a Shimadzu TOC 5000 soluble C analyzer. Soil pH was determined in a 1:2 soil:0.01 M CaCl₂ suspension (Thomas, 1996). Particle size distribution was determined by hydrometer (Gee and Bauder, 1986). Ultra-
violet spectrophotometric analysis was performed on all samples and flavonoid standards using a Beckman DU650 spectrophotometer.

**Paired Knapweed / Native Plot Field Study**

Two study plots were established the first week of June, 1996 at the interface of a knapweed infested / non-knapweed (native species) area on the slope of Mt. Sentinel, Missoula, MT. Plots were 15 x 30 m with a 4 m buffer area between. The plots were divided into a grid of 450, one meter sub-plots. Ten sub-plots were chosen at random from each plot (native and knapweed infested) and one Idaho fescue plant was selected for resin capsule placement within each native species sub-plot and one spotted knapweed plant was selected for resin capsule placement within each knapweed infested sub-plot.

A soil probe was used to remove a 6 inch soil core at a 45° angle from the rhizosphere of each plant. Polyester capsules containing 1100m² surface area of Ambersorb 563 non-ionic carbonaceous resin were placed in the bottom of the cavity left by each core and watered in with 60 ml of de-ionized water. The soil core was then replaced over the capsule and another 40 ml of de-ionized water was poured over the area. Bulk soil samples were collected from each sub-plot where resin capsules were placed and brought to the lab for resin capsule placement and incubation.

In the lab, 50 g fresh weight of each bulk soil sample was placed into an incubation jar, a resin capsule was placed on the sample and another 50 g fresh weight of soil was used to cover the capsule. Soil was then moistened with 10 ml de-ionized water, the jars were sealed and placed in a 30° C incubator. Jar weights were monitored gravimetrically for water loss.
Field plot capsules received two 100 ml allotments of de-ionized water at 5 and 10 days after resin capsule placement. Capsules remained in place in the field and in the incubation jars for 14 days after which they were removed and transported to the lab for extraction and analysis. Five composite soil samples were collected from the rhizosphere area of each treatment (fescue and knapweed) and also transported to the lab for analysis at this time. Twenty-five grams of these samples were placed in 50 ml of 0.4 M K₂SO₄, shaken for 30 minutes and then filtered through 1.2 μm glass fiber filter paper. Water, methanol and K₂SO₄ extracts were analyzed as described above. Water and methanol extracts were analyzed for total phenol content using Prussian blue method (Price and Butler, 1977). Total soluble C was analyzed in the water extracts using a Shimadzu TOC 5000 soluble C analyzer.

Experimental Field Plot Study

Research plots were established at the University of Montana experimental gardens at the base of Mt. Sentinel, Missoula, MT in the summer of 1996. The field was plowed to a depth of 30 cm and disked in at a depth of 15 cm. A 6 x 12 m area was determined as the study site and 32 one meter square plots were measured out in a 4 x 8 m matrix with 0.5 m buffer strips between each plot. Each plot was excavated to a depth of 15 cm, the soil sifted through a 1 cm² diameter mesh screen and mixed with approximately 3 kg Eko-Kompost. Native palouse prairie (blue-bunch wheatgrass, lupine, yarrow and Idaho fescue), spotted knapweed, and vegetation free, bulk soil plots were established in a completely randomized design. Seeds of each species were sown.
into compartment flats and grown for approximately 12 weeks in a greenhouse under natural light regime until time of transplant into the study area. Field plots were divided into 100 1 dm² square sub-plots into which seedlings were transplanted. The native Palouse prairie plots were planted with 25 each of the four species listed above placed in the sub-plots in a completely randomized design. One hundred knapweed plants were transplanted into each of the knapweed plots. The vegetation free / bulk soil plots were maintained by hand weeding and light hand-tilling.

Plants were established for one year prior to resin capsule placement in June 1997. Capsules containing 1100 m² surface area of Ambersorb 563 non-ionic carbonaceous resin were placed into the rooting zone (as described above for the paired knapweed / native field plot study) of 4 randomly selected Idaho fescue plants in the native palouse prairie plots and 4 randomly selected spotted knapweed plants in the knapweed plots. Four capsules each were also placed in the no vegetation / bulk soil plots. Capsules were left in place for 10 days after which they were removed and transported to the lab for extraction and analysis. Capsules were extracted using a constant drop rate from a buret set to deliver a two-step sequential rinse: 25 ml de-ionized water and 25 ml 50 % methanol (Chapter 1). Each fraction was filtered through a 1.2µm glass fiber filter paper and stored at −10 C° until analyzed. Water and methanol extracts were analyzed for soluble hexose sugars using the anthrone reactive carbon method and for total phenol content using Folin-Denis. Total soluble C was analyzed in the water extracts using a Shimadzu TOC 5000 soluble C analyzer.
Ultra-violet spectrophotometric analyses were performed on all samples and flavonoid standards using a Beckman DU650 spectrophotometer. Samples were also roto-evaporated down and re-suspended in a 60:40:1 mixture of methanol:water:acetic acid, compounds separated on high performance liquid chromatography (HPLC), using a reverse phase column, and analyzed by ultraviolet and refractive index detectors.

Statistical analysis

One-way ANOVA was used to determine differences in ARC and TOC between the different species and the no-plant soil control for all studies. Tukey’s HSD was used to determine mean separation with an alpha level of 0.05.

RESULTS

Greenhouse study:

There were no significant differences in soluble hexose sugars at alpha level 0.05 for aqueous extracts (F=1.74, p=0.195) and 100% methanol extracts (F=0.84, p=0.442) between spotted knapweed, Idaho fescue and bulk soil (Figure 1). For the 50% methanol extract spotted knapweed showed significantly higher (F=4.78, p=0.0171) hexose sugar levels compared to both the Idaho fescue and the bulk soil (Figure 1). Although there was no significant difference in TOC levels between spotted knapweed and Idaho fescue knapweed tended to have higher levels of TOC in the aqueous extract. Bulk soil TOC was significantly higher (F=4.61, p=0.0190) than both the knapweed and fescue (Figure 2).
With ARC values standardized to root biomass, spotted knapweed exhibited significantly higher levels of soluble hexose sugars for aqueous extracts ($F=55.19$, $p=0.0001$), 50% methanol extracts ($F=14.91$, $p=0.0038$) and 100% methanol extracts ($F=26.92$, $p=0.0006$) when compared to Idaho fescue (Figure 3). Total organic carbon levels were also significantly higher ($F=9.00$, $p=0.0149$) in spotted knapweed when the values were standardized to root biomass (Figure 4). Percent ARC:TOC was much higher in the spotted knapweed than in the Idaho fescue and bulk soil (Figure 5).

**Paired Knapweed / Native Plot Field Study**

Spotted knapweed exhibited almost five times as much ($F=4.54$, $p=0.0410$) ARC in the aqueous extraction compared to Idaho fescue (Figure 6). Total organic carbon values were significantly higher ($F=11.72$, $p=0.0001$) in the spotted knapweed aqueous extracts compared to Idaho fescue (Figure 6). Similar to the greenhouse study, ARC:TOC tended to be higher in the knapweed than in the fescue (Figure 5).

**Experimental Field Plot Study**

There were no significant differences at alpha level 0.05 ($F=1.60$, $p=0.2501$) for the ARC values of the aqueous extracts in the 1997 field plot experiment. Spotted knapweed and bulk soil had similar levels of hexose sugars with Idaho fescue tending to show slightly lower levels (Figure 7). For the 50% methanol extracts knapweed exhibited significantly ($F=22.36$, $p=0.0002$) more ARC than both the Idaho fescue and bulk soil (Figure 7). There was no statistical difference between the fescue and the bulk soil.
soil although the fescue showed a trend towards higher ARC levels in the 50% methanol extract.

Total phenolics were negligible across all studies and there were differences between treatments. Neither the Prussian blue nor the Folin-Denis methods were sensitive enough to detect low levels present in the samples. The u.v. absorbance data and HPLC data indicate that there are u.v. active compounds present in the rhizosphere of knapweed that are different than those found in the Idaho fescue and bulk soil samples (Figure 8). These data also indicate that the retention times of these compounds from the knapweed are similar to that of flavonoid standards.

**DISCUSSION**

Ambersorb 563 non-ionic carbonaceous resin capsules appear, from the results of preliminary research, to effectively sorb rhizosphere C and allow us to differentiate between the chemistry of the rooting zone of spotted knapweed and Idaho fescue. We propose that the ability of this capsule to be inserted into and removed from plant rhizospheres with little disturbance to the roots and associated microbial communities may help plant and soil scientists to investigate the importance of rhizodeposition products in plant/plant competitive interactions. As stated previously, other methods, to date, are unable to effectively capture rhizodeposition products *in situ*.

The relatively short duration (10-14 days) that the capsules were left in the ground may have an effect on the types of compounds that are sorbed to the resin. If the capsules were left in the ground for a longer period of time, perhaps compounds of a higher molecular weight would be adsorbed to the resin (Johns and Skogley, 1993). Further
work is needed to test the ability of resins to sorb compounds of different molecular weight over varying lengths of time.

The preliminary data indicates that there is significantly more ARC and TOC in the rhizosphere of spotted knapweed when compared to Idaho fescue. Perhaps the rooting zone of spotted knapweed presents a preferred living environment for microbial populations in the soil. The readily available sources of C found in higher concentrations in the rhizosphere of knapweed compared to Idaho fescue may produce a chemical gradient which actually enables knapweed to 'steal' microbial populations from neighboring plant communities. In a competitive environment, an active rhizosphere microbial community could benefit associated plants in a variety of ways. Microbial populations: decompose organic matter and mineralize organic forms of N, P and S (Tate, 1995); increase availability of Cu, Fe, Zn and Mg through release of chelating agents (Marschner, 1996); contribute to biological N fixation (free-living, associative and symbiotic); promote plant growth through production of plant hormones; and improve soil physical characteristics (Tate, 1995). Any of these could result in knapweed out-competing native plant species.

Importantly, most of the ARC extracted from the resin capsules placed in the rhizosphere of spotted knapweed in the greenhouse study was eluted by the 50% methanol rinse indicating that the materials sorbed to the resin were not just simple hexose sugars (Figure 3). The ability of the methanol to effectively remove these sorbed compounds and the presence of both carbohydrates and UV absorption indicates that sugars found in the rhizosphere of knapweed are likely glycosides associated with polyphenolic compounds, perhaps glycosilated flavonoids. Glycosilated flavonoids are a
15 carbon compound with a basic C6-C3-C6 structure made up of two aromatic rings linked by a three carbon ring with hydroxyl groups attached (Markham 1982). These hydroxyl groups provide a place for attachment of one or more sugars. The anthrone reagent (in ARC analysis) hydolyzes the glycosidic linkages and reacts with the 6 C sugars present and therefore does not discriminate between free sugars and glycosides.

Root exudates can act as possible signals to attract beneficial root symbionts such as *Rhizobium* (Phillips, 1992; Hungria and Stacey, 1997) and certain mycorrhizal fungi (Ratnayake et al., 1978; Becard et al., 1992; Chabot et al., 1992; Poulin et al., 1993). Phenolic compounds, including glycosilated flavonoids, released by plant roots have been shown to induce transcription of nodulation genes in N₂ fixing bacteria (Phillips, 1992; Hungria and Stacey, 1997) and some flavonoids, particularly flavanols, have been found to stimulate germination and increase hyphal elongation of certain arbuscular mycorrhizal (AM) fungi (Becard et al. 1992, Chabot et al. 1992, Poulin et al. 1993). Our results indicate that there may be such materials being released into the rhizosphere of spotted knapweed which may increase it’s ability to attract beneficial microbial communities.

Further research could be conducted with NICR’s in the rhizosphere of spotted knapweed and Idaho fescue using an alternative resin type (Amerlite XAD-7, see Chapter I) to effectively identify the compounds that are being sorbed to the resin. Once specific compounds are identified, it may be easier to determine how these compounds may influence seedling germination and growth and how this, in turn, may affect competitive interactions between spotted knapweed and Idaho fescue.

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FIG 1. Resin extracts of Anthrone reactive carbon for water, 50% and 100% methanol fractions for knapweed, fescue, and bulk soil in the 1996 greenhouse experiment.

FIG 2. Total soluble organic carbon values for aqueous resin extracts from knapweed fescue and bulk soil in the 1996 greenhouse experiment.
FIG 3. Resin extracts of Anthrone reactive carbon standardized with root biomass for water, 50% and 100% methanol fractions in the 1996 greenhouse experiment.
FIG 5. Anthrone reactive carbon as a percent of total organic carbon for knapweed, Idaho fescue and soil for the paired native/knapweed field study, greenhouse study and the experimental field plot study.

FIG 6. Anthrone reactive carbon and total soluble organic carbon values in the paired native / knapweed field study.
FIG 6. Anthrone reactive carbon and total soluble organic carbon values in the paired native / knapweed field study.

FIG 7. Anthrone reactive carbon values for water and 50% methanol extracts for knapweed, fescue and soil in the experimental field plot study.

FIG 8. Chromatogram for HPLC separation of resin extract from knapweed, fescue and bulk soil using a C-18 reverse phase column, 50:50:1 (water, methanol, acetic acid) mobile phase and a UV detector.

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LITERATURE CITED


