Native plants as sentinels of tritium contamination

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NATIVE PLANTS AS SENTINELS OF TRITIUM CONTAMINATION

by

Colleen A. Grant

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A thesis submitted in partial fulfillment
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ABSTRACT

Native Plants as Sentinels of Tritium Contamination

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This study assessed the potential of six plant species growing in the Mojave Desert, as sentinels of tritium contamination below earthen caps covering radioactive waste. The plants, grown in hydroponic tanks and 3 m columns, were evaluated for treatments representing three different levels of tritium contamination. Tritium, a radioactive isotope of hydrogen, replaces a hydrogen atom in a water molecule and readily migrates through soil. Plant roots coming into contact with soil moisture do not discriminate between tritiated water molecules and dihydrogen water molecules in the uptake process. Plants have the potential to be more effective monitors of radioactive waste-sites than mechanical sensors, because roots sample a larger soil volume. Tissue collection from plants to detect tritium can be completely aboveground, offers the option of transpiration capture or biomass analysis, and potentially exposes technicians to lower levels of radioactivity than encountered during installation and maintenance of in-ground mechanical sensors.
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Tritium in the Environment

Tritium, the radioactive isotope of hydrogen, is naturally produced in small quantities when molecules of oxygen or nitrogen interact with cosmic radiation. Anthropogenic sources such as weapons production and fission of uranium fuel in nuclear reactors for electricity generation have greatly increased the quantities of tritium in the environment; peaking in 1963 due to above ground testing of nuclear weapons (Scanlon 1992). The predictable rate at which tritium decays into helium and the emission of a beta particle in that decay process make tritium useful for numerous scientific and commercial applications. For example, the analysis of samples (for tritium) from aquifers thought to contain only water thousands of years in age, has proven infiltration by current precipitation into ground water is taking place; proving the potential of contaminating important water sources by modern surface-dispersed pollutants. Snow that accumulated on a glacier after 1944 can be dated to the year of snowfall by the amount of tritium incorporated into the water molecules comprising the snow.

Research in the biological sciences and the modern medical treatment of human illnesses exist at their present level of sophistication in part because of the use of
radioactive tracers and therapeutic techniques. Replacement of hydrogen atoms in organic compounds with tritium can be specifically directed to produce labeled DNA, RNA and proteins for in vivo metabolic studies. Tritiated compounds have been used to paint dials of watches, clocks, compasses, electron tubes, gaseous tritium light devices (GTLDs) and other products with widespread commercial and industrial applications (Hicks et al., 2000). Beneficial as these applications may be, they create toxic waste materials that must be carefully segregated at the end of their usefulness to special permanent storage containers at low-level radioactive waste sites.

Burial sites for low-level (radioactive) waste are located on government reserves such as Los Alamos, New Mexico and Hanford, Washington where nuclear research is conducted and at commercial sites designed as repositories for waste from medical, university and industrial sources. The earliest sites were located in the eastern United States where there is significant recharge of ground water sources due to annual precipitation rates. Because a tritium atom can easily replace a hydrogen atom in a water molecule and migrate through the environment, the designers of these sites reasoned that burial trenches should be located in clay layers relatively impervious to water movement. When filled to capacity, the trenches were ‘capped’ with the original excavated soil and left to the ages by the trench designers who felt secure in their knowledge that the radioactive waste was safely entombed until sometime in the distant future when the radioactive material had decayed.

Subsequent changes in government regulations required monitoring of sealed burial trenches which led to the discovery that significant tritium migration was occurring out of the trenches. It was found that tritium generally did not leak from the bottom of
the trenches as the clay layers prevented such movement in accordance with the original design. When precipitation fell onto a cap, it was conducted downward into the trench by infiltration, gradually filling the trench. Inundation of the stored waste caused the water to become contaminated with tritium, creating a contamination plume when the water eventually migrated out over the upper edges of the trench through surface soil pervious to water, a condition that was dubbed the 'bathtub' effect (Meyer 1979).

Since high annual precipitation and impervious soils have been identified as contributing significantly to the problem of environmental contamination, it has been reasoned that the low annual precipitation characteristic of arid lands would provide an acceptable solution to the problems encountered at the storages sites located in the eastern United States (Nativ 1991). Storage site design was modified as storage sites were constructed in the arid southwestern United States. At Los Alamos, New Mexico, shafts were bored into volcanic tuff, waste generated by government research projects was inserted and concrete plugs used to seal the holes. At Beatty, Nevada trenches were constructed in soil that would permit drainage from the trench bottom in the 'unlikely' event of infiltration. Follow-up monitoring, performed in compliance with Federal nuclear safety regulations, found tritium migration at both sites suggesting that factors other than soil permeability and annual precipitation rates should be considered when designing low-level radioactive waste sites (Andraski 1996). The arid lands of the western United States are subject to extreme precipitation events that can deliver enough moisture in one storm to damage and infiltrate an earthen cap composed only of the original native soil, therefore the timing and
quantity of rainfall events must be taken into consideration when designing trenches and their earthen caps. Careful consideration of waste site geology is necessary as tritium was found in the vapor phase moving from the shafts into the surrounding area associated with faults in the tuff blocks at Los Alamos (Purtyman 1973).

Cap Closure and Root Growth

Competition by plants for growing space is typically so intense that a bare patch of soil with the requisite nutrients and moisture will remain uncolonized for only a short time. Unless maintained plant-free by weeding and herbicide use, the earthen caps of burial trenches will be colonized. The roots of these plants can open channels in the soil permitting infiltration of precipitation into the trench, particularly after root decomposition (Devitt and Smith 2002). In addition, roots of phreatophytes may extend into the trench, contact radionuclide-contaminated water and then translocate radionuclides into adjacent areas. During the growing season, évapotranspiration by plants will remove more water from the upper soil layers than can be removed through evaporation from a bare soil (Nylan et al., 1990), potentially reducing trench infiltration. Under conditions of mass flow, tritium ($^3$H) attached to a water molecule exhibits behavior similar to protium ($^1$H) with root water uptake, followed by water movement into the xylem with subsequent transpiration export from the leaf in the vapor phase. Interestingly, in contrast to flow through the xylem, diffusional flow in intracellular water transport constitutes only 1% of plant water movement. Therefore, most tritiated water follows the mass flow pathway from the soil through a plant, then to the atmosphere (McFarlane 1979). The percentage of tritium that becomes
incorporated into the organic molecules of a plant (through exchange of carboxyl and hydroxyl hydrogens) varies greatly as the process is strongly dependent on the tritium concentration of the aqueous solution, duration of exposure to the tritiated source, the length of residency (of the tritiated aqueous solution) within a plant as governed by photosynthetic/transpiration activity, and the availability of exchange sites in DNA, RNA, carbohydrate molecules and proteins (Garland and Ameen 1979, McFarlane 1979, Dinner et al., 1980, Belot 1986, Smith and Ziegler 1990, Diabaté and Strack 1993, Kim and Baumgärter 1994, Strack et al., 1995, Baumgärter et al., 2001)

Cap Design

Of the several components needed to limit water movement in earthen caps, one modern design includes several layers of rock, graduated in size, to discourage trench penetration by roots (Nylan et al., 1990). Design components also take advantage of plant water-removal capacity to prevent radionuclide migration by using plants to remove infiltrating precipitation from the upper soil layer of the cap before water can move deeply enough to contact waste. Establishing ground cover can reduce erosion of the earthen cap by wind and surface movement of water. Choosing indigenous plants has several advantages; amendments to the upper soil layer of the earthen cap need not be extensive as the plants are already adapted to the local soil conditions, increasing the probability of establishing a succession pattern that will eventually permit the burial site to blend seamlessly with the surrounding undisturbed vegetation. Landscaping an earthen cap with exotic plants, having the capacity to thrive under the local growing conditions, sets up the potential for further ecosystem destruction by
species that may naturalize and become invasive, although this is less likely in an arid environment than in more mesic regions. The plants can also be used as monitors for movement of tritiated water in soil vapor or liquid phase by sampling transpiration or cuttings of plant tissue.

Statement of the Problem

While low-level radioactive waste sites can be monitored with mechanical sensors, plants have been used as contamination monitors and offer several advantages. Plant roots can sample a larger volume of soil than mechanical sensors and require far less maintenance once established. Aboveground tissue collection to detect tritium contamination offers the option to capture transpiration or biomass, and potentially exposes the technicians to lower levels of radioactivity than encountered during installation and maintenance of in-ground mechanical sensors.

The purpose of the hydroponic study was to assess the potential of six Mojave Desert plant species as sentinels of tritium contamination below earthen caps that cover sealed radioactive waste. We hypothesized that each of the six desert species could absorb tritiated nutrient solutions, displaying differing biomass accumulations in response to species-specific rooting characteristics and treatments. In the hydroponic experiment we grew *Larrea tridentata* (creosotebush, an evergreen shrub), *Bromus madritensis* ssp. *rubens* (red brome, an exotic annual grass), *Pleuraphis rigida* (big galleta, a C₄ perennial grass), *Ephedra nevadensis* (Mormon tea, a leafless shrub), and *Atriplex canescens* (four-wing saltbush, a C₄ shrub) in hydroponic tanks to identify species that could be recommended for monitoring purposes based on amount of...
transpiration capture and tritium activity in tissue samples. Tritium activity was estimated by liquid scintillation counting. In the shallow growing conditions of the hydroponic tanks, the most rapid translocation of tritium from nutrient solution to tissue to atmosphere via transpirational stream was predicted for the species whose natural rooting phenology consisted of producing numerous lateral roots rather than development of a single, central tap root. The numerous shallow, lateral roots would give greater surface area for absorption and therefore, greater potential for uptake of tritiated solution. Thus *Pleuraphis* with its average of 38 roots per small clump, extending to 0.1 m (Nobel 1981) was predicted to have a more rapid uptake than *Ephedra* with its characteristic reliance on one primary tap root and deeply penetrating secondary roots (Wallace and Romney 1972).

Our second hypothesis was that the quantity of tritium moved through the plant would be treatment and species dependent, based on interacting and species-specific factors such as root absorption area, leaf/stem area available for photosynthesis, transpiration rate (related to stomatal density and photosynthetic rates) and the level of tritium activity in the respective treatment. Thus a species with a large absorptive root area, large leaf area for photosynthesis and grown in the 1000 tritium unit solution (T.U.), was predicted to have the greatest tritium activity in the transpiration samples. On the basis of leaf area, *Pleuraphis* (with leaf blades 2-6 cm in length and culms 5-8 dm) and *Atriplex* (leafs: 1.5-5 cm length, 2-8 mm wide) was predicted to have higher transpiration tritium activity than *Larrea* (ovate, 5-10 mm length) or *Ephedra* (leafless) (Munz 1974).
In the column study, based on the principal that roots are able to absorb soil moisture from a large soil volume by creating water potential gradients, our third hypothesis was that our two most vigorously growing species (as identified by the hydroponic study) in addition to our invasive phreatophytic species, would absorb and translocate a tritiated source of water introduced to the root system, at a depth of approximately 1 meter. *Larrea tridentata, Atriplex canescens* and *Tamarix ramosissima* (salt cedar, a C₃ phreatophyte) were grown in 3-meter, soil-filled columns to identify which species would respond with the strongest tritium signals. The column height permitted development of root systems analogous to field conditions (Wallace *et al.*, 1974, Lee and Laurenroth 1994, Smith *et al.*, 1997, Bowerman and Redente 1998, Yoder and Nowak 1999, 2000) permitting us to identify root response to tritium treatment under conditions more similar to field conditions than in the hydroponic study.
CHAPTER 2

LITERATURE REVIEW

Sources of Tritium

As a radioactive isotope of hydrogen, tritium originates from natural and man-made sources. The interaction of atmospheric oxygen or nitrogen molecules with cosmic radiation creates approximately 69 megacuries year$^{-1}$ of tritium (Greenfield 1971, Scanlon 1992). Prior to the 1962 treaty banning aboveground testing of nuclear weapons, it was estimated that more than 1700 megacuries of tritium were released with most deposited into the stratosphere (Greenfield 1971, Moghissi and Carter 1971). Tritium levels in precipitation increased several hundred times above normal and remained elevated, caused by a half-life of 35-40 days in the troposphere for tritium (Greenfield 1971). Fission of uranium fuel in nuclear reactors for weapons production and the generation associated with the production of electricity has been a major source of tritium produced in both the water and gaseous forms (Crowson 1971, Greenfield 1971). Tritium is also produced when boron and lithium are bombarded with neutrons in processes designed to control pH and reactivity in nuclear reactors (Crowson 1971). Neutron reactions with helium or deuterium, in reactors using heavy water, produce prodigious amounts of tritium (Crowson 1971).
As an emitter of soft beta particles, tritium has unique properties that are utilized in a variety of scientific and medical applications. Replacement of hydrogen atoms in organic compounds with tritium can be specifically directed to produce labeled DNA, RNA and proteins for \textit{in vivo} metabolic studies (Feinendegen 1971, Chevolleau \textit{et al.}, 1993, Kusheva 1995, Jia \textit{et al.}, 1996). Tritium is used to hydrogenate polymerized plastic compounds that are mixed with fluorescent zinc sulfide crystals to produce luminescent coating compounds (Seelentag 1971). Dials painted with these compounds have been used for watches and clocks (Seelentag 1971, Hicks \textit{et al.}, 2000). Compasses, electron tubes, and gaseous tritium light devices (GTLDs) are products with widespread commercial and industrial applications (Kisalu \textit{et al.}, 1991). At the end of their usefulness, these products are placed in permanent storage at low-level radioactive waste sites; the shallow burial site subsequently sealed-off beneath an earthen cap. The surrounding environment, however, can become contaminated with radionuclides originating from waste sites moving by capillary action (Perkins and DePoorter 1985) if local soil, plant and atmospheric conditions are not properly considered (Meyer 1979, Hicks \textit{et al.}, 2000). Robinson and Gronow (1996) compared tritium levels in leachates from thirty United Kingdom landfills with calculated activities based on historic levels and decay rates. All leachate samples contained concentrations of tritium much higher than anticipated from landfills specializing in industrial wastes. Samples of leachates taken at three municipal waste sites in the midwestern United States had activities ranging from 225 tritium units (T.U.) to over 8000 T.U. when precipitation activities at these sites were 50 T.U. or less. [Notes: 1 tritium unit is equal to 3.2 picocuries per liter. Current EPA
regulations define a tritium exposure of 20,000 pCi/L as yielding a 4 mrem/y dose, the maximum contaminant exposure per year (EPA 2002). The likely sources of tritium were determined to be luminescent paints and luminescent instrument dials (Hackley et al., 1996).

Tritium migration out of storage sites is also an ongoing problem at U.S. government sites. Storage shafts cut into rhyolite tuff ashflows at the Los Alamos, New Mexico facility were filled with waste in 1966 and they were discovered to be leaking tritium in 1970. Water moving through the joints of the fractured ashflows (in liquid and vapor phases) became contaminated with tritium via the replacement of protium by tritium atoms on water molecules naturally occurring in the tuff. The contamination then moved westward away from the trenches in both liquid and vapor phases (Purdyman 1973). At the Oak Ridge National Laboratory, an average annual precipitation of 132 cm combined with short vertical distances to ground water sources resulted in a strongly contaminated leachate containing $^3$H, $^{60}$Co, $^{90}$Sr and other nuclides. The problem was further exacerbated by short lateral distances between trenches and surface drainage features such as tributary streams, thus creating an inadequate distance of sorptive soil and rock for nuclide capture before contact with water sources (Webster 1979).

Advantages of Arid Lands Waste Sites

In the United States, storage sites in arid regions characterized by low annual precipitation, extensive unsaturated zones and large potential evapotranspiration rates were selected to limit waste plumes (Meyer 1979). These criteria were chosen in
response to problems encountered at waste sites in more mesic regions. Commercial
deposition of low-level radioactive waste in shallow land disposal sites began in the
1960's at locations in the eastern states, where most waste was generated (Meyer
1979). Sites were chosen for hydrological and geological features, such as low soil
profile permeability to prevent migration of radionuclides. Trenches were excavated
and waste was placed into the trenches, the trenches were then covered with the
original excavated soil. By the mid 1970's sampling at the West Valley site in New
York and the Maxey Flats site in Kentucky revealed that infiltration of the earthen
caps was occurring, and that precipitation was contributing to the downward
movement of radioactive waste (Meyer 1979). Microorganisms, present in the soil of
the earthen cap, utilized the organic waste materials as metabolic substrates, creating
acids and other leaching agents. The combination of precipitation, acids (organic and
inorganic) and complexed radionuclides created a hydraulic head, enabling the
leachate to move laterally and downward from the trenches (Meyer 1979).

Disposal of low-level radioactive wastes originating from any source other than
national defense activities became the responsibility of each state when Congress
passed the Low-Level Radioactive Waste Policy Act of 1980 (Siefken 1982). The
infiltration problems encountered at the eastern sites prompted the Nuclear Regulatory
Commission to refine regulations governing site selection (Siefken 1982). For
example, the potential for water to enter the waste site has to be minimized by
placement of trenches only in well drained areas that are not flood-prone, or with
upstream drainage into the storage area; ground water level has to be below trenches
to minimize, and preferably prevent, contact between waste and ground water (Siefken
1982) because migration by radioactive waste will most likely occur through movement of contaminated ground water (Nativ 1991). The difficulty of meeting these requirements led to recommendations for arid region storage sites where low annual precipitation has the potential for limiting transport of radioactive isotopes (Meyer 1979, Scanlon 1992). Winograd (1981) advocated waste sites in the Great Basin region, where unsaturated zones can be several hundred meters deep in contrast to more mesic areas where such zones are thin or non-existent (Nativ 1991). The precipitation regime in the Great Basin region creates conditions where water flux is generally very small (Winograd 1981); direct infiltration penetrates only into the top few meters of soil (Nativ 1991). Another advantage of arid lands is the sorptive capacity of the soil for any radioactive leachate that does occur (Winograd 1981).

Placement of radioactive waste in the thick vadose zone of an arid region also requires that extreme precipitation events associated with deserts not be ignored; otherwise migration of radioactive leachate may still occur. Properly-engineered earthen caps are needed to prevent surface water from infiltrating the trenches and subsequently carrying radioactive wastes into ground water by percolation. Cap subsidence and cracks from desiccation were found to permit surface water to infiltrate waste trenches at the Beatty, Nevada disposal site for low-level radioactive waste (Robertson 1980). Capillary barriers in conjunction with plantings of native vegetation have been recommended to effectively reduce infiltration (Nyhan et al., 1990, Anderson et al., 1993). This combination of transpiration and evaporation is more efficient in reducing infiltration than a bare earthen cap, where moisture is removed from the upper soil profile by only evaporation. Plant roots have the
potential to proliferate in the entire soil profile, removing water by transpiration from a greater volume of soil than evaporation (Anderson et al., 1993). Over a six year period, Waugh et al. (1994) found that vegetated earthen caps maintained seasonally consistent levels of soil water volume. Below 2.25 m (rooting zone), soil water volume increased beyond the removal capacity in trenches covered by bare earthen caps, leading to recharge (deep drainage). Nyhan et al. (1990) at Los Alamos, New Mexico evaluated caps constructed in four layers, the top three layers (capillary barrier) increasing in coarseness with depth (71 cm topsoil, 46 cm gravel, 91 cm river cobble) and the final layer consisting of 38 cm sandy silt backfill to a depth of 38 cm. The test caps and the comparative, standard-design caps (20 cm sandy loam topsoil and 108 cm sandy silt backfill) were planted with blue grama and western wheatgrass to ascertain which design most effectively prevented infiltration of precipitation into the simulated trenches. The experimental caps had twice as much biomass as the standard caps, attributable to the capillary barrier enhancing moisture content of the topsoil layer by hindering downward percolation. The greater biomass of the experimental caps, through evapotranspiration, removed 96% of precipitation received by the experimental cap compared to 88% removal on the standard cap. Another benefit of this experimental cap was the reduced potential for translocation of radionuclides to the surface by plant roots as the gravel-cobble layer effectively barred root extension into the sandy silt backfill layer, the layer that would be in contact with stored waste (Nyhan et al., 1990).

In the cold desert ecosystem where the Idaho National Engineering Laboratory is located, Anderson et al., (1993) evaluated the impact of topsoil on the storage of
precipitation received during seasons of plant dormancy. An ideal earthen cap should have sufficient depth to hold received precipitation; during the growing season, the vegetation should remove all stored water, thereby preventing the possibility of water infiltrating the waste interred below (Anderson et al., 1993); results indicated the topsoil layer should be at least 2 m thick. In this study, four plant species were grown to determine species capacity to remove soil water: Agropyron desertorum (crested wheatgrass, naturalized bunchgrass), Elymus lanceolatus cultivar ‘Sodar’ (streambank wheatgrass, naturalizing varietal), Elymus cinereus (Great Basin wildrye, native bunchgrass) and Artemisia tridentata (Big sagebrush, long-lived perennial). At the end of two years, E. cinereus and Agropyron reduced soil moisture to about 10%; Artemisia and E. lanceolatus required 3 years to produce enough root mass to achieve similar results. Evaporation from the bare soil control trenches reduced soil moisture to 20%.

A five year test of capillary barriers at the U.S. Department of Energy Hanford Site (Washington) using lysimeters to compare vegetated barriers with non-vegetated barriers (Glendon et al., 1997) showed that vegetated caps were more effective in moisture removal even when inundated with three times the normal level of precipitation. The non-vegetated caps were unable to store the extreme moisture levels and drained. The suitability of Artemisia tridentata, Chrysothamnus nauseosus (rabbitbrush) and twelve varieties of grasses were also evaluated. They found several advantages for using vegetation on earthen caps: more effective soil water removal, stabilization of the cap against wind or water erosion, and the root growth lowered soil
bulk density consequently enhancing hydraulic conductivity and ease of water infiltration to decrease surface runoff.

Problems in Waste Site Monitoring

Following closure of a waste trench, Federal and state regulations require that migration of radionuclides must be monitored (Andraski 1997). The monitoring process can be expensive for monitoring entities with limited funding; this was the case at Texas A & M University where budgetary constraints required an economical design for an abandoned landfill previously utilized by the university and since converted to a discharge channel servicing a sewage treatment plant and fire-fighting school (Riland et al., 1996). Monitoring wells should have been standard protocol with such a situation but because the drilling costs for monitoring wells were prohibitive, an alternative approach was developed. Because a monitoring plan requires identifying the radionuclides stored in the waste trench and the presence/absence of gamma-emitters, a pressurized ionization chamber was used to survey the surface area of the trenches. Soil samples and water samples were collected in the discharge channel, and then analyzed for gamma and/or beta emissions. Riland et al. (1996) also mentioned other low-cost monitoring alternatives, such as hand-held low-exposure ratemeters and solar stills to collect tritiated condensation.

At arid zone waste sites, subsequent monitoring after closure is especially critical, as the hydrological studies conducted prior to excavation of a waste trench may generate data different than that obtained from post closure studies. One source of
error can come from using the excavated native soil to refill the trench and then assuming that the excavated soil retains the hydraulic properties of the undisturbed soil (Andraski 1996). A study at the Beatty, Nevada Waste Facility found the excavated soils became more homogeneous, whereas the uppermost layers of undisturbed soil are different in texture, acting as a natural capillary barrier against infiltration of surface water. Exposure to air reduces moisture content of the excavated soil, drying it, thus enhancing the prospect of vapor flow. This is problematic because vapor flow was documented as the most significant downward transport mechanism in the unsaturated zone during the summer, and failure to account for the difference in the filler soil could lead to inaccurate infiltration estimates (Andraski 1996 and 1997, Scanlon 1992).

Monitoring for leakage can be accomplished by utilizing a network of closely-spaced mechanical sensors that require frequent attendance by technical personnel for maintenance and sampling. The extremely low soil water potentials attained by desert soils during drought periods are problematic for commonly-used sampling devices such as tensiometers (Andraski 1997). Lysimeters may inaccurately portray moisture movement in undisturbed soil due to changes in soil characteristics related to disturbance of soil profiles during installation (they are expensive to construct) (Allison et al., 1994). Thermocouple psychrometers have been successfully used to measure the ability of desert plants to lift water from lower soil levels (Yoder and Nowak 1999), psychrometers are functional under water potentials of ≈ -0.2 to -8 MPa (Andraski 1997). According to Andraski (1997), the disadvantages of using psychrometers as waste site monitors are that psychrometers require careful
installation, precise calibration and collected data is often difficult to interpret. Accurate calibration of psychrometers requires prior determination of the water potential range that will be encountered to select the correct calibration solution, and selection of operating parameters such as optimum cooling current or cooling-current duration. Data interpretation is also dependent on correct assessment of probable water potentials, as the cooling current determines the microvolt output used to calculate water potential in the area of soil being sampled (by the psychrometers). Also critical to attaining accurate water potential data is determination of whether an entire curve or a datum point best represents the microvolt plateau associated with equilibrium between evaporation at the sensing junction and relative humidity of the sensing chamber (Andraski and Scanlon 2002). The cost of maintaining sufficient mechanical sensors to provide accurate monitoring can be significant and prohibitive when the cost of manpower is factored into a budget (Entry et al., 1996). Andraski (1997) used 100 thermocouple psychrometers installed at distances of 1 m with 0.35 m spacing on four simulated waste trenches constructed near Beatty. One-fourth of the monitored psychrometers (25 out of 95) provided no useable data as water potentials at their locations were outside of the instruments' operational range (greater than 8 MPa). Comparing vegetated trenches with non-vegetated trenches at a depth of 0.6-0.75 m, Andraski (1997) concluded the inefficiency of psychrometers placed in vegetated trenches was due to the soil water potential being drawn below 8 MPa by plant roots depleting available soil moisture in the root zone. He further concluded evaporation was not the causation as water potentials in non-vegetated trenches remained within the operational range of the psychrometers. Brown and Johnson (1976), Brown and
Collins (1980), and Fischer (1992) reported significant drift in thermocouple psychrometers used less than three years after being placed in service, requiring removal for recalibration; this form of monitoring may not be suitable for long-term, undisturbed monitoring. Andraski (1997) reported inaccuracies in water content estimates occurred when neutron attenuation was used to detect water content in the immediate vicinity of waste storage drums, but did not state the precise nature of the problem. Nyhan et al. (1990) reported the use of a neutron probe to estimate soil water storage in experimental earthen caps (radioactive waste was not present) however, no problems were encountered. The use of time domain reflectometry (TDR) to monitor low-radiation waste sites was not encountered in the literature; therefore TDR is not discussed in this thesis.

Plants as Sentinels of Detection

The benefits of using tritium as a tracer for radioactive leachates (Striegl 1988, Hackley et al., 1996) can be extended to monitoring water movement in unsaturated arid zones to quantify the processes of net moisture flux and solute transport (Andres and Egger 1985, Scanlon 1992, Allison et al., 1994, Beyerle et al., 1999, Gupte et al., 1999, Rangarajan and Athavale 2000, Le Gal La Salle et al., 2001). One technique uses the peak anthropogenic production reached in 1964 and an assumption of a steady-state system to estimate the downward rate of penetration for precipitation (Scanlon 1992). In Bolivia, historical data of $^3$H concentrations in precipitation, and a correlation between increasingly tritiated rainfall as latitude and distance from the
ocean increase, was used to estimate the ground water resources available to a
growing city (Stimson et al., 1996).

To a plant there is little biological difference between H₂O and tritiated water
(Kalisz et al., 1988), allowing use of tritium as a hydrological tracer to identify the
plant water source(s) (Bishop and Dambrine 1995). One of the earliest studies to
apply tritium to follow water movement was performed by Woods and O’Neal (1965)
on oak trees in South Carolina. Tritiated water was applied to a sandy soil at three
depths; trees ranging from 2.1-2.4 m distant from the injection site were sampled for
transpiration. They found the uptake rate from the top 30 cm was 38 times greater
than uptake from 60-90 cm layer; this degree of reliance on surface roots for water
was greater than Woods and O’Neal (1965) anticipated. Capitalizing on the vertical
gradient present in these South Carolina forest soils, Bishop and Dambrine (1995)
applied ³H and ¹⁸O in two different strata. The tracers showed Scots pine (Pinus
sylvestris) had an average uptake depth of 12 cm while Norway spruce (Picea abies)
had an uptake depth of only 3 cm. During the 1950’s in Scotland, forestation project
managers planted Sitka spruce (Picea stitchensis) forests in areas not traditionally
forested; forests that 30 years later were being devastated by windthrow. The spruce
seedlings were planted above a peaty gley soil having little aeration, resulting in poor
root development as the trees grew, making them vulnerable to high winds. Under
economic pressure to resolve the problem, drainage ditches were cut to drain excess
water from the forest soil with the goal of improved soil aeration. Boggie and Knight
(1980) tested the remedial drainage effectiveness by applying tritiated water on the
ground surface and at the mineral layer surface. If the drainage system as established
was effective, root mass would have increased in the soil level where tritium was
applied due to better aeration of the soil, resulting in increased uptake of the tracer.

Based on the amount of tritiated solution recovered in soil samples, Boggie and
Knight (1980) concluded the drainage was ineffectual. In a study by Lewis and Burgy
(1964), wells were used to inject tritiated water into a zone of saturation beneath a
ground water source suspected to be the summer uptake source of oak trees in
California. Tritium activity was found in leaves collected from oaks growing
downslope of the injection wells, verifying that oaks can deploy roots extensive
distances through the cracks in a fractured rock system to reach a water source.

European studies such as the one performed by Ibrahim et al. (1982) on Pinus pinea
reported results similar to the various American studies.

The accidental discovery of a contamination plume when testing plant transpiration
for tritium activity in Kentucky revealed the potential of plants as monitors for
radionuclide migration. A Rickard and Kirby (1983) study at Maxey Flats Waste
Disposal Site, Kentucky used sap water of deciduous trees to detect contamination
from atmospheric releases of tritium vapor. In a subsequent study, Rickard and Kirby
(1987) discovered a data anomaly in transpiration samples collected from one tree.
This led to the discovery of two trees exploiting a small contamination plume moving
through the rock strata, which led to the subsequent installation of several monitoring
wells. Kalisz et al. (1988) designed a follow-up study to evaluate the usefulness of
trees as contamination monitors. The results defined a complicated hydrological
profile with extensive horizontal movement in the underlying fractured sandstone
formation. Tritium activity in leaf water displayed a seasonal component, increasing
during the summer when the trees began relying on deeper, contaminated water sources as surface moisture sources were depleted.

Stringer et al. (1989) sought to quantify the relationship between predawn xylem water potential and uptake of tritiated water from subsurface sources contained in the fractured sandstone underlying Maxey Flats, Kentucky (low-radiation waste site). As in the two previously described studies, a seasonal correlation between vertical rooting depth and exploitation of specific moisture sources was observed; the tritium activity in transpiration samples and samples of transpiration plus leaf sap increased as trees relied progressively more on the subsurface water sources. As the summer progressed, oaks that were able to reach tritiated water in the fractured sandstone maintained higher water potentials than the more shallow-rooted hickories that were unable to reach the tritiated water sources. The water potentials revealed that the oaks and two hickories suffered less water stress than most of the hickories and two oaks that were unable to exploit the tritiated water. The primary conclusion of the study was that when surface water sources were available, white oaks exploited that source first; exploitation of deeper water sources increased as surface sources became increasingly depleted.

Accurate prediction of the evaporative fluxes (Allison et al., 1994, Andraski and Prudic 1997) that impact movement of tritiated water (Purtyman 1973, Schulz et al., 1991, Scanlon 1992, Andraski 1996, 1997) requires comprehending the important role of desert plants in the hydraulic profile of the unsaturated zone in arid regions. On vegetated earthen caps and adjacent areas, evapotranspiration has been shown to remove almost all precipitation in the soil (Allison et al., 1994, Waugh 1994) through
the vast root systems typical of plants indigenous to non-riparian desert ecosystems (Freckman and Virginia 1989). Utilizing plants to monitor the movement of tritiated water offers an efficient and economical alternative to sensors as a root system generally has the capacity to sample large volumes of soil. On the Nevada Test Site, research measured the amount of tritium transported annually from interned waste to the atmosphere through transpiration. The results indicated shrub maturation was linked to a strong trend of increased tritium translocation (Schulz et al., 1991). Using a weighing lysimeter, Sammis and Gay (1979) measured an evapotranspiration loss of 259 mm for a mature Larrea over an 1-year period with measured precipitation of 234 mm. Evaporative loss from adjacent bare soil plots was calculated as 231 mm using a water balance budget; evapotranspiration loss from adjacent Larrea in-ground plants was estimated as 242 mm (Sammis and Gay 1979). Smith et al. (1995) investigated the water balance of perennial shrubs growing in three types of geomorphic surfaces commonly found in the Mojave Desert. The total annual evapotranspiration was calculated for each surface type, with an overall average of 129 mm year	extsuperscript{-1}, plant transpiration was responsible for 35% of the soil water loss. Sala et al. (1996) found that some Tamarix have evapotranspiration rates at almost two times the potential evapotranspiration rate when temperature and vapor pressure deficit combine to create conditions of high evaporative demand. A 1999 site assessment performed by Bechtel Nevada sampled Larrea tridentata, Salsola kali and an Atriplex species for tritium concentration in plant water and in transpiration. The sampling was performed as part of long-term monitoring at Radioactive Waste Management Sites on the Nevada Test Site; the presence/absence of tritium activity in Larrea plant water and
transpiration samples identified the location of tritium migrating from a large inventory (Bechtel Nevada 2000). Data from soil-gas monitoring and lysimeters indicated tritium migration was vaporous; infiltrating precipitation moved no deeper than 30 cm, not reaching ground water sources. Continued plant monitoring was recommended, animal monitoring would be initiated only if tritium activity in vegetation increased sufficiently to warrant the change in monitoring protocol (Bechtel Nevada 2000). Using solar distillation to obtain Larrea plant water samples, Andraski et al. (2002) tested a new extraction method to purify the samples for scintillation counting. Constituents of the plant water that could interfere with scintillation counting were adsorbed on a graphite-based solid-phase-extraction column; comparison to the standard toluene extraction method was favorable. Andraski et al. (2002) concluded the extraction column method was simpler, more cost effective than-and as accurate as toluene extraction. These studies have established that more evapotranspiration takes place on earthen caps vegetated with desert species than on caps kept denuded of vegetation, desert plants are able to utilize tritiated moisture sources and that desert plants are valuable monitors of tritium movement in the environment.

Studies Describing Tritium Interactions in Plants

Radionuclide-laden gases are released during the daily operation of nuclear plants, resulting in the atmospheric exchange of a protium atom for a tritium atom in water vapor molecules, creating HTO (hydrogen-tritium-oxygen) molecules (Kahn et al., 1979). HTO can enter soil through gaseous diffusion, followed by dissolution in soil
water or by the process of rainout (incorporation in raindrops during cloud formation) and washout (raindrops contaminated by contact with a tritium plume during movement between cloud and ground) (Murphy 1993). The tritiated soil water can again become HTO through the mechanism of plant transpiration (Raney and Vaadia 1964, Murphy 1993). This cycle stimulated a large body of research in the United States, Europe and Russia to discover what effects exposure to tritiated water (HTO) vapor has on plants and animals. This relationship between tritiated water in the vaporous state, tritiated water in the soil and plant transpiration will determine the activity of tritium available to desert plants utilized for monitoring purposes at waste sites, thereby governing the effectiveness of monitoring regimes. Raney and Vaadia (1964) grew Helianthus and Nicotiana under lights and exposed the plants to tritium by adding the isotope into nutrient solutions. The activity of tritium in leaf petioles and stem tissue reached equilibrium (with nutrient solution activity of tritium) in twelve hours. Seeds, floral parts, and growing leaves did not reach equilibrium even when plants were grown in tritiated solution from germination to senescence; the lack of equilibrium in leaf tissue was attributed to transpirational processes. Belot et al. (1979) exposed grape leaves to HTO vapor in spring and autumn to develop and quantify a model capable of predicting plant contamination following exposure to HTO vapor. A ratio of tritium activity in plant tissue water to the activity of tritiated water vapor in the air is the basis of the model, with the ratio influenced by factors such as quantity of water in leaf tissue per unit area, resistance to vapor flow through stomata and exposure time. The authors demonstrated that the ratio was a decreasing function in relation to decreasing temperature, therefore in accordance with Henry's
Law, decreasing temperature will produce a decreasing constant when the solution concentration of a solute is divided by its concentration in the air (vapor pressure) (Belot et al., 1979). Garland and Cox (1982) found the observed diffusion rate of HTO through the boundary layers of leaf and stomata in bean leaves agreed with the predicted rates of a model developed by Belot (1979). Couchat et al. (1983) examined HTO vapor exchange in *Helianthus annuus*, and found that illumination increased the rate of exchange. Labeling with $^{14}$CO$_2$ and $^3$H confirmed HTO vapor can participate in exchange reactions after assimilation into tissue water (Guenot and Belot 1984). Brudenell et al. (1997) assessed the validity of the STAR-H3 model used by the UK Ministry of Agriculture to evaluate consumption risk for human food crops exposed to HTO. They measured the time required for cabbage, lettuce and soil to clear HTO contamination through the processes of vapor exchange and transpiration for the fast component of the model. This component consisted of plant tissue water and organically bound tritium in exchangeable positions of organic molecules; the slower model component was tritium bound in non-exchangeable positions. They found that while 95% of HTO was removed from tritium-exposed plants in 6 hours; stem and root decontamination required over 48 hours; several times longer than the 6-hour rate predicted by the model (Brudenell et al., 1997). Ichimasa et al. (1999) measured the rate woody plant leaves and roots, herbaceous plant leaves and roots, lichens, mosses and soil oxidized tritium gas to tritiated water. Lichens, mosses and roots performed oxidations more quickly than leaves of the woody and herbaceous species. The oxidation activity was postulated as resulting from the hydrogenase activities of bacteria colonizing vegetation surfaces, inferring lichens, mosses and roots have larger
populations of hydrogenizing microorganisms than herbaceous and woody plants. Stewart et al. (2001) developed a conceptual model to predict the rate at which fruit exposed to tritiated vapor would be able to lose HTO.

Several authors have published papers describing tritium movement in soil and plants. Koranda and Martin (1973) contributed a paper that summarized the work of other researchers investigating tritium movement in ecosystems and then gave details of supporting experiments conducted in their own lab. Some of the conclusions highlighted in the paper were that when rainfall quantities are above 0.1 cm/d, translational movement is more important to tritium movement through the soil than diffusion, but in quantities below that amount, diffusion is more important. The paper emphasized that compared to other components of an ecosystem, tritium will have the longest half-life in soil and that will lengthen the half-life of tritium in plants withdrawing soil moisture from that soil due to continuing exposure. Koranda and Martin (1973) also suggested decay of tritium activity in plants took place in three stages, the initial stage where 90% of tritium activity was lost in the transpirational stream through stomatal chamber flushing occurred in 0.9 hour. The second stage was attributed to removal of tritium activity from water-related metabolic processes or biochemical processes and required approximately 17 hours. The third phase had a half-life of 270 hours, accounted for 0.2% of the total tritium activity and was related to organically bound tritium that had been translocated from areas of photosynthate production to root and stem tissue. McFarlane et al. (1979) focused on tritium movement in plants, describing pathways by which tritium may enter plants, giving an overview of isotopic fractionation in plants. They listed several cautions about
experimental techniques and interpretations such as biological damage attributed to the decay of $^3$H $\to$ He, where tissue damage may actually be caused by free oxygen radicals. Belot (1986) listed studies exploring mechanisms of tritium transfer in tissue water and subsequent incorporation into organic matter. Diabaté and Strack (1993) described organically bound tritium (OBT) in animal and plant ecosystems, defining exchangeable tritium, isotopic effects, specific activity ratios, and kinetic rates.

Emphasizing the difference in mass between protium and tritium, Murphy (1993) wrote a review paper focusing on environmental transport of tritium, detailing which chemical, physical and biological processes are most influential in moving tritium through terrestrial, aquatic and atmospheric systems. A system of equations modeled the transferal of tritium between soil, vegetation and the atmosphere by interaction of washout, soil water content, infiltration of precipitation, and rooting characteristics of vegetation. An explanation was given for the observation that at nuclear production sites, tritium activity in vegetation water is generally higher than tritium activity in atmospheric water vapor due to continuous enrichment of soil water by oxidization of atmospheric tritiated molecular hydrogen. The majority of the paper reviewed, in detail, the mechanisms that move tritium through specific systems, distinguishing between the terrestrial environments of soil, vegetation and animal, then an integrated explanation of mechanisms governing the cycling and transport of tritium in terrestrial environs. Aquatic systems were likewise partitioned into freshwater, ocean and aquatic life for detailed explanations and then an integration of mechanisms responsible for tritium movement was detailed.
Using potted maple, elm and oak trees Amano and Garten (1991) tested a model predicting $^3$H in leaf water following uptake of atmospheric tritium vapor (HT). The best results were found when models incorporating atmospheric and inground sources were used. In a study to discern the primary contributor of tritium vapor in a deciduous forest at Oak Ridge, Amano and Garten (1992) found plant transpiration was the source of HTO during the active growing season and deriving from soil evaporation during the dormant season. The soil at the study site (Oak Ridge National Laboratory) was tritium contaminated; during the growing season, plants absorbed tritiated water through their root systems and released the tritiated water as HTO. Amano et al. (1995) examined the effects of sustained atmospheric release of tritium on plants during a twelve day test at Chalk River Laboratories, Canada. Soil microorganisms converted the HT to tritiated water (HTO) which was quickly used by plants growing in the plots. The amount of organically-bound tritium (OBT) gradually increased, never achieving a steady state. Amano (1995) also studied the response of plants to tritiated methane, which is produced during the detonation of nuclear bombs, in nuclear fusion reactors and through microbial action at waste sites. $C_3$, $C_4$ and CAM plants were exposed to atmospheric tritiated methane. Tritium accumulated in tissue water and organic matter in $C_3$ and $C_4$ plants, no accumulation was found in the CAM plants, indicating that the substitution of a tritium atom for a hydrogen atom did not occur during photosynthesis in CAM plants (Amano 1995). Proposed mechanisms for plant acquisition of hydrogen from tritiated methane require the mediation of microorganisms living on the plants and the conversion of $CH_3T$ to HTO, methane/methanol oxidation to CO$_2$ or oxidation of methane to aldehydes or formic
acid. The authors proposed that the aldehydes and formic acid are sufficiently small to
diffuse into leaf cells where they would be metabolized in biochemical pathways.
Another proposed mechanism was incorporation of tritium during the dark reaction of
photosynthesis where an intermediate methane derivative from the light reaction
interacts with CO$_2$ molecules to produce tritiated water and tritiated photosynthates
(Amano 1995).

Noting tritium has a larger mass than hydrogen, Garland and Ameen (1979)
examined what effect larger mass might have on the incorporation of tritium into
organic matter. Since most plant water derives from uptake processes rather than
catabolic processes, they reasoned that any difference in isotopic composition between
environmental water and plant water results from a physical process. They concluded
an isotopic effect led to discrimination against tritium incorporation during
photosynthesis. Belot et al. (1983) used double labeling of $^3$H and $^{14}$C to determine the
linear correlation coefficient for the specific activity ratio of $^3$H:$^{14}$C (0.97). They
concluded all tritium extracted from tissue samples had been incorporated through
photosynthesis, but the quotient of total $^3$H specific activity to organically bound
specific activity was significantly less than unity, suggesting an isotopic
discrimination.

Klobe (1972) found an effect of age on the rate of tritium removal. In this study,
seven-week-old corn plants had a residual signature of 6.4% at 1 week, 1.8% at 2
weeks and 0.4% at 3 weeks. At thirteen weeks, the leaf tissue still retained 19% of the
label two weeks after tritium was put into the growing medium. Goncharova et al.
(1976) injected fruit-bearing strawberry plants with tritiated water to study water

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movement. Under well-watered conditions, unripe fruits acquired a radioactive signature faster than ripe fruit. Under droughted conditions, water transfer to ripe fruit was greater. Using calamondin plants, Mantell et al. (1980) found evidence of isotopic discrimination in the transfer of water from pedicel to fruit; compared to soil, the pedicel reached a 60% labeling whereas fruit reached 20%. The fruit also required a longer period to clear radioactive signature than leaf tissue following a chase experiment. Diabaté and Strack (1997) found tritium incorporation rates correlated with the availability of photosynthetically active light.

Several studies have focused on exchange processes between tritium and hydrogen atoms in water and biomolecules. In an elegant study, Kim and Baumgärtner (1994) exposed maize and barley seedlings to tritium-laced nutrient solutions in a steady state system, permitting determinations of the specific activity ratio for tritiated tissue water and organically-bound tritium. In comparison to the nutrient solution, tissue water was enriched in the barley seedlings (C\textsubscript{3}) and depleted in the maize seedlings (C\textsubscript{4}). Under a steady state system, barley had a higher transpiration rate than maize, favoring tissue water enrichment. In a follow-up study, Baumgärtner and Kim (1995) focused on resolving the contradictory results several studies had found regarding the kinetics of organic incorporation; suggesting isotopic fractionation inherent to the methods of tritium analysis are responsible, specifically, freeze drying samples to remove water was mentioned. Again using barley and maize models, the study demonstrated stable OBT increases more rapidly than photosynthetic processes that incorporate hydrogen. They proposed two processes, one involving isotopic fractionation in the photosynthetic pathway and another involving exchangeable
hydrogen positions in proteins and DNA to account for the derived growth function. Baumgärtner and Kim (1997) collaboration found tritium was incorporated more readily than hydrogen in non-photosynthetic reactions when they measured the incorporation rates of hydrogen and tritium during growth rate and growth increment studies. Baumgärtner et al. (2001) found the in vivo incorporation of tritium into exchangeable hydrogen positions is thermodynamically more favorable than photosynthetic incorporation. Hydrogen bridges are structural components of biomolecules such as carbohydrates, proteins, DNA and RNA; the hydrogens are also polar covalently bound to carbon, nitrogen and other component atoms of the biomolecules. In comparison, hydrogen bridges between water molecules are stronger so the quantum mechanics principle of uncertainty predicts tritium will move from water to the weaker bond in biomolecules. Photosynthetic processes discriminate against the larger mass of the $^3$H atom, favoring the smaller $^1$H atom; this is the kinetic isotope effect. The movement of tritium from a water molecule, replacing hydrogen occurs in microseconds; linkage of peptide chains and protein folding requires milliseconds thus Baumgärtner et al. (2001) found tritium incorporation into maize resulted in a non-exchangeable OBT growth increment 2.4 times greater than the growth increment of hydrogen. They concluded this thermodynamic isotope effect compensates for the discrimination exerted by the kinetic isotope effect, explaining why observed tritium incorporation rates are inconsistent with predicted rates when only the kinetic isotope effect is considered as the operating mechanism.

Many studies have used tritium as a tracer to measure transpiration in plants. Kline et al. (1970) placed tritiated water into boreholes drilled in three trees indigenous to
the lower montane rainforest of Puerto Rico. They based calculated activity rates on the concept of radionuclide dynamics in a steady-state system where inflow equals outflow. This principle and technique were the basis for delivery of tritiated solution to the root systems of plants grown in the column study (described in this thesis). Kline et al. (1976) measured transpiration rates in Douglas fir (Pseudotsuga menziesii) by injecting tritium solution in a series of holes around the bole of a tree and to their surprise, established that the peak tritium activity in collected twigs was the same at all levels of the canopy. They postulated that either the xylem path to the upper branches was less tortuous than previously thought or the greater amount of sunlight hitting the upper canopy stimulated greater photosynthesis with an attendant greater water demand than lower canopy levels. Regardless of the mechanism, their findings allowed us to sample at any location in the canopy of our plants, confident that the tritium activity would be the same. Jordan and Kline (1977) found a strong correlation between sapwood area and transpiration rates for understory and canopy trees in an Amazonian rainforest. In the column study we grew Tamarix ramosissima, a phreatophytic tree, with excellent potential as a tritium sentinel. Knowing a correlation exists between sapwood and transpiration rates, suggests a potential method of calculating translocated tritium activity in the event Tamarix roots encounter a contamination plume. Young et al. (1970) developed a technique to collect transpiration from the pad of Opuntia, a genus of cactus. The relationship between atmospheric humidity level and degree of activity in leaf tissue water was described by Mantell et al. (1979); drier air encouraged equilibrium between tritium levels in leaf water and nutrient solution. In trees, Luvall and Murphy (1982) found
that the distance from the injection site must be considered when calculating transpiration rates, and the simple technique of collecting transpiration water in clear plastics bags (tied to a branch) to be reliable. Sansigolo and Ferraz (1982) used tritium to compare seasonal differences in transpiration rate in *Pinus caribaea*.

McIntyre (1994) supplied tritiated water to the roots of *Avena* and established a link between transpiration and guttation by coleoptiles.

*Zeng et al.* (1996) developed an ecosystem model based on thirty years of reclamation data taken from projects designed to stabilize drifting sands in a desertified region of northern China. The amalgamation of non-indigenous plants failed to develop a sustainable ecosystem; degeneration of succession in the ecosystem prompted numerous experiments that revealed water balance and water transfer were the two most important processes governing interactions between soil, atmosphere and plants in this region. In the opinion of *Zeng et al.* (1996), the complex relationship between plant water balance, the physiological characteristics of the desert plants, and the ecology of the desert ecosystems ruled out using simple means of quantitative analysis for developing an ecosystem model that would be the basis for future environmental management decisions. *Zeng et al.* (1996) developed a model of interacting atmospheric-, plant-, and sand metabolic pools where the soil area utilized by the root mass of a plant was defined as a cylinder. Tritium concentration-time curves derived from daily transpiration capture was used to calculate daily mean transpiration rates from steady-state and non-steady state systems. It was suggested that the resulting data could be used to predict which plant species would have a water balance compatible to the environment.
The natural production of tritium, the large quantities of tritium produced by nuclear bomb testing and nuclear plant operations, and the effectiveness of use in biological and geological research as an isotopic tracer, have produced an extensive body of scientific literature. Tritium-related research continues to be a ‘hot’ topic in areas as diverse as plasma edge physics (Federici et al., 2003), tumor angiogenesis (Shimamura 2003), estimating residency time for water in New Zealand catchments to better understand the influences exerted by landscape features (McGlynn et al., 2003) and the diffusion properties of HTO in saturated rock (Palut et al., 2003). These four studies, and dozens of others published in 2003, serve to illustrate the fact that tritium-contaminated waste products continue to be generated. Tritium waste products will require long term storage that must be monitored, as mandated by nuclear regulatory agencies, for radionuclide migration. Monitoring will include the use of plants as sentinels of contaminant migration.
CHAPTER 3

MATERIALS AND METHODS

Preparation of the Greenhouse

To prepare the UNLV greenhouse for the use of radioactive isotopes, several steps were taken. Because tritium readily migrates through concrete, the interior block walls of the greenhouse bay were painted with epoxy paint. To contain spills or leakage from the hydroponic tanks, two tables in this interior bay were fitted with containment trays. Each tray was constructed from a 1.22 m x 2.44 m sheet of plywood with 5.08 cm x 10.16 cm sides, and then coated with fiberglass and resin. The trays were tested several times for water tightness; they were filled with water, leaks observed, leak locations marked and more fiberglass and resin added until the trays proved to be watertight.

Hydroponic Study

The hydroponic tanks were constructed of PVC pipe with an average interior diameter of 20 cm; tanks were approximately 28.5 cm in height (average volumetric capacity: 8 liters). Custom-made bottom plates cut from sheet PVC were glued with PVC glue to each section of pipe. Removable caps were also cut from sheet PVC to provide closed-system hydroponic tanks. The space between the interior wall of the tank and the ridge of the bottom plate was swabbed with extra glue to establish a
barrier against leakage. The bottom seam was sealed on the exterior and interior with clear silicon to provide another barrier layer against tritium leakage. Three holes, 2.5 cm in diameter and equally-spaced, were bored in each lid to permit insertion of three plants per tank. One hole, 0.65 cm in diameter, was bored in each lid to permit insertion of 0.635 cm flexible tubing used for aeration. A 1 cm hole was bored into each lid to permit the placement of a pH probe into the tank for daily adjustment of the solution pH. A rubber cork was used to seal the hole.

Large rubber corks were bored out to a minimum width of 1 cm and used to hold the plants upright in the holes of the lid. One-hundred percent cotton cosmetic pads were cut into rectangles approximately 1 cm by 6 cm in size. One strip was wrapped around the stem of each plant to hold the plant upright in the cork hole and to also act as an evaporative barrier. The depth of wrapping was decreased as plant stems enlarged and wrapping was discontinued when the plant stem reached sufficient size to totally fill the bored-out hole in the cork.

As proper temperature is crucial for optimal root functionality, the experiment was conducted in a temperature controlled glasshouse bay. To further insulate the root systems against the high temperatures incident with summer in the University of Nevada, Las Vegas glasshouse, each tank was double wrapped with thermal barrier material (a commercially available bubblewrap sandwiched between two layers of aluminum foil) to achieve an insulation value of R19. Each tank was then wrapped in a reflective layer of white, plastic shelf-liner placed over the thermal barrier to reduce thermal absorption. Each tank lid was similarly insulated. To further reduce heat absorption, the top of each cork was painted with a glossy white spray paint. In July,
early morning temperature of the nutrient solution in each tank varied from 24 °C to 27 °C, but by late afternoon the temperature of the nutrient solution approached 33 °C. To decrease thermal absorption a canopy constructed of shade cloth on a PVC framework was erected over each table. The shade cloth was pulled back during the morning, allowing full sunlight to reach the plants and in the afternoon the shade cloth was draped over the framework to reduce the heat load being imposed on the hydroponic tanks.

The nutrient solution in the tanks was a modified Hoagland's solution at one-half strength (Jones 1997). The solution pH was checked daily with a digital pH meter and initially adjusted to pH 7.0 using either 1M H₂SO₄ or a 10% KOH solution. Two weeks after transfer of plants into the hydroponic tanks, the target pH for each tank was revised downward to 6.8 in an effort to ameliorate iron chlorosis. Prior to the addition of tritium, the nutrient solution was changed on a weekly basis to maintain optimum nutrient levels. Half-strength Hoagland's solution was made with reverse osmosis water and stock nutrient solutions each week. A submersible pump connected to a 10-m length of 1.9 cm flexible tubing was used to deliver nutrient solution from the mixing tank to each hydroponic tank.

Aeration of the nutrient solution in each tank was accomplished with nine deep-water aquarium pumps. Each pump was attached to a 5-way air control valve by an 8 cm long section of 0.64 cm clear aquarium flex tubing. Five tanks, consisting of one tank per plant species, was attached to each pump using 0.64 cm clear aquarium flex tubing with length being dependent on distance from the pump. The aeration on each
tank was adjusted daily by manual manipulation of the appropriate valve on the 5-way air control valve until a gentle bubbling of the solution surface was observed.

Seeds for the experimental plants were obtained from the following sources: *Larrea tridentata*, *Bromus madritensis* ssp. *rubens*, *Pleuraphis rigida* and *Ephedra nevadensis* were collected at the Desert FACE (Free Air Carbon Enrichment) Facility located on the Nevada Test Site (36°49' N. 115°55'W. 970 m). *Atriplex canescens* seeds were obtained from a commercial seed company (Western Native Seed Company, Coaldale, Colorado) because seeds collected at the FACE site were not viable. Seeds were germinated in a standard greenhouse mix of peat:sand:vermiculite and grown in the UNLV glasshouse. *Larrea, Pleuraphis, and Ephedra* are slow-growing perennials and required approximately nine months of growth in soil to reach a size judged sufficient to survive stresses associated with transfer to the hydroponic tanks. *Atriplex* exhibited a much faster growth rate and reached suitable size in three months. *Bromus* was planted four weeks prior to the projected date of transfer. While growing in soil, seedlings were watered daily with a dilute Hoagland's solution.

The initial step in preparing a plant for transfer was to place plant and pot into a bucket containing reverse osmosis water to a depth slightly below the rim of the pot. The plant was left in the bucket for at least 30 minutes to totally saturate the soil solution. The pot was then removed and the root ball was immersed into another bucket of water to remove most of the soil. The plant was then placed in a flat wash pan where a gentle stream of reverse osmosis water was used to dislodge remaining soil fragments. Care was taken to preserve as much of the root mass as possible. After washing, the plant stem was wrapped with a strip of cotton padding, and inserted
into a bored-out cork. The cork and plant were then fitted into the plastic lid set into a hydroponic tank filled with nutrient solution.

_Larrea, Ephedra, Pleuraphis_ and _Atriplex_ were grown in the hydroponic tanks for 3.5 months and _Bromus_ for two weeks. Due to the weight of a filled tank, it was decided rotation of the tanks around the bay to compensate for possible microclimatic effects was too great a risk due to the possibility of dropping a tank. Following the establishment of viable root systems in the nutrient solution (new roots are distinguishable as being white in contrast to the dark, suberized roots) two levels of tritium activity were introduced. For each species, three tanks were given a treatment corresponding to 100 tritium units and three tanks were given a treatment corresponding to 1000 tritium units; for a total of six tanks per species and five species. [Note: 1 tritium unit is equal to 3.2 picocuries per liter.] The total number of experimental tanks was 45.

A tritium-labeled water solution was pipetted into each tank beginning at sunrise on Day 1 (Julian Day 232). The exact volume of solution to be introduced was calculated based on desired level of radioactive activity and volumetric capacity of the individual tank. Tissue and transpirational sampling began on Day 1 (Julian Day 232), one hour post-introduction of tritium. Samples were then collected every two hours until sunset. On Day 2 (Julian Day 233), samples were collected every two hours beginning at sunrise and ending at sunset. On Day 5 (Julian Day 236), one midday sampling was done to check for residual tritium in transpiration and biomass accumulation. One milliliter aliquots of nutrient solution were taken from each tank for determination of residual radioactive signature. Individual plant biomass was
separated into above ground and root biomass, each section was placed into a pre­weighed heavy-duty polyethylene bag and weighed. The open bags were then spread out on tables in the greenhouse for three weeks to air dry the biomass. The biomass plus bag were then weighed again to determine the dry biomass weight.

To determine the amount of transpiration taking place on the whole plant level, 1 tank for each treatment and each species were weighed at each sampling time. To prevent root systems from drying out during the week, measured amounts of nutrient solution were added on a daily basis. The amount added to each individual tank varied according to transpiration activity of the tank. Total transpiration by the plants of an individual tank was calculated as: the initial weight measured on Day 1 (Julian Day 232) minus the final weight of the tank (measured on Day 5), plus the weight of any nutrient solution added during the week.

Transpirational capture and plant tissue were assessed for radioactive signature. Polypropylene scintillation vials (20-ml in volume) were numbered, pre-weighed and the weights recorded for both sampling techniques. For transpirational capture, 15 x 15 cm plastic bags (Woods and O’Neal 1965) had a 2.5 cm wide x 15-cm long strip of rock wool inserted. The bags were individually numbered, weighed and weights recorded. To accomplish the transpiration capture, a bag was placed over a plant, tied in place with narrow cotton cord and left in place for five minutes. At the end of the sampling period, the cord was cut, the bag carefully removed to prevent sample contamination by organic material, twisted shut and sealed into a labeled, pre-weighed vial. Tissue sampling took place after the transpirational capture. Approximately 0.2 g of leaf material and adjoining stem were removed from the parent plant with a razor.
blade, using a different blade for each treatment level to minimize cross-contamination. Each tissue sample was sealed into a labeled, pre-weighed vial.

Transpiration sample vials were weighed while still sealed to establish any weight gain attributable to transpiration capture. Each vial was then opened and 5 ml of Beckman Ready Safe liquid scintillation fluid added to the vial. The plastic bag was lacerated with a scalpel to permit contact between radioactive transpirational capture and the liquid scintillation fluid. Five milliliters of Beckman Ready Safe was then added for a total of 10 milliliters. The vials were allowed to sit for one week to permit dissolving of the plastic bag by the liquid scintillation fluid. The vials were dark adapted for 24 hours prior to counting for 5 minutes (Beckman LS 6500).

Tissue sample vials were weighed while still sealed to establish difference in weight between empty and filled vials. Samples were cut into smaller pieces (samples were not removed from the vial) and 1 ml of Beckman 450 tissue solubilizer (quaternary ammonium hydroxide in toluene) was added to the vial. The vial was tightly recapped and placed into a water bath (45°-50° C) until the plant tissue dissolved. The liquid digest was separated from the tissue sample by decanting. The quantity of decant obtained was approximately 1 ml. As the deep green coloration caused by chlorophyll in the tissue samples had the potential to produce inaccurate scintillation counts through color quenching (Lee 1980, Burrell and Brunt 1981), each sample was clarified by the addition of 0.5 ml hydrogen peroxide (H₂O₂) to the vial. To quench chemiluminescence 70 μl of glacial acetic acid was added to the sample. Nine milliliters of Beckman Ready Protein liquid scintillation cocktail was added to
the vial to reach a total liquid volume of 10 ml. The vials were dark adapted for 24 hours prior to counting (Beckman LS 6500 scintillation counter).

To investigate the possibility of isotopic discrimination during evaporation of nutrient solution from a hydroponic tank (water loss directly to the atmosphere, not through plant transpiration), an evaporation test was performed. Beakers with 100 ml of 100 T.U. or 1000 T.U. (in water) were placed in UNLV greenhouse. Evaporative losses were measured daily and a 1 ml aliquot taken to ascertain tritium activity. Comparison was made between the initial tritium activity and daily activity for the finding of no isotopic discrimination due to evaporative processes.

Data were analyzed with ANOVA and multiple regression techniques to determine significant differences between the treatments (SigmaStat 2.0, SPSS, Inc.)

Column Study

Twenty-seven columns, each 305 cm in length and 10 cm in diameter, were used for this portion of the experiment. The columns were installed in a custom-made wood cabinet designed for the dual purposes of support and to shade the lower section of each column. The upper section of each column was wrapped in an insulation blanket to reduce fluctuation in soil temperatures. Columns were constructed of a clear plexiglass upper section and a lower opaque section made of white PVC. The plexiglass section was wrapped in black polyethylene plastic to prevent solar radiation from discouraging root growth at the soil-plexiglass interface. Other columns were one-piece, cut from 10 cm PVC pipe. Based on growth responses of the hydroponics study, *Larrea tridentata* and *Atriplex canescens* were selected and planted into
eighteen columns, with nine columns per species. Nine *Tamarix ramosissima* (saltcedar) from a previous study were brought into the greenhouse to also be part of the column study. During establishment, seedlings were initially watered with a dilute Hoagland's solution. Columns were placed in barrels filled with water to a depth of 45 cm. For the phreatophytic *Tamarix*, columns were perforated in the lower 20 cm to simulate a groundwater source.

Approximately two months after seedlings were transplanted to the columns, the black polyethylene plastic was partially removed to determine the extent of root development. When root development greater than 1 m was observed, bore holes were drilled into the columns and an entry port installed. The entry port consisted of 3.81 cm PVC elbow, glued to a straight piece of (3.81 cm) PVC pipe, 7.6 cm in length. The entry port was inserted into the column via the 7.6 cm section of 3.81 cm PVC pipe, then held in place with a thick layer of silicon sealer. Soil samples were collected at the entry site prior to installation of the entry port.

For each species, treatments consisted of 100 tritium units (n = 3), and 1000 tritium units (n = 3); for a total of six columns per species, totaling 18 columns. The entry port was sealed with a parafilm cap to prevent evaporation of the tritiated water. One hour after tritium was introduced to the rooting zone, sampling for transpirational capture and tissue accumulation of tritium began (Day 1 treatment, Julian Day 257), following the sampling protocol previously outlined for the hydroponic study, plants were sampled every two hours until sunset for 2 days. One midday sampling was done to check for residual tritium in transpiration and tissue accumulation on Day 8 (Julian Day 264). Soil samples were taken prior to installation of the tritium entry.
ports and when the columns were sectioned at the end of the column study. A liquid extract was made to detect radioactive signature in the soil. A 1.5 ml microcentrifuge tube was weighed, 1 g of soil was placed into the tube, 1 ml of double-distilled H$_2$O added to the tube; samples were mixed on a vortex. The tube was microcentrifuged for 10 minutes at 5000 RPM. The supernatant were consistently within $\pm$ 0.1 ml of 1 ml in volume. Several soil samples obtained during post-experiment sectioning of the columns were saturated, resulting in supernatant samples slightly greater than 1 ml in volume; 9 ml of Beckman Ready Safe liquid scintillation fluid was added, vials were dark adapted for 24 hours and counted (Beckman LS 6500).

The aboveground biomass was harvested from each column, inserted into pre-weighed heavy-duty polyethylene bags, and weighed. The open bags were placed on tables in the greenhouse to air dry for three weeks and then re-weighed to determine the dry weight of the biomass. The columns were sectioned following harvest of all aboveground biomass from the columns. Soil and root samples were obtained at 17 cm above the entry port, at the entry port, 17 cm below the entry port, and at the bottom of the column. Soil samples were treated as described above and root samples were treated as described above for tissue samples.

Data were analyzed with ANOVA and multiple regression techniques to determine significant differences between the treatments (SigmaStat 2.0, SPSS, Inc.)
CHAPTER 4

RESULTS OF HYDROPONIC AND COLUMN STUDIES

Hydroponic Study

Growth Response of Roots and Shoots

The shoot biomass of *Atriplex canescens* increased rapidly after transfer to the hydroponic tank, developing lignified stems greater than 1 cm in diameter. Three plants developed stems 1.9-2.1 cm in diameter. In 5 of the 9 tanks, one of the three plants had noticeably less vigorous growth. On the *Bromus madritensis ssp rubens* plants, new leaves began growing within 1 week after transfer. Shoot biomass increased slowly in *Ephedra nevadensis*, with maximum stem diameter of 0.5 cm at harvest. *Larrea tridentata* grew slowly with most of the plants being limited to 5-7 new leaf-pairs for the entire hydroponic growing period. In *Pleuraphis rigida* shoot biomass approximately doubled during the hydroponic growth period.

There was a significant difference in shoot biomass (p = <0.001) between *Atriplex* and the other species, but not between the other species. On a per plant basis the *Atriplex* shoot biomass was fifty-three times greater than the next largest plants of *Ephedra* and more than a hundred times greater than the other three species (Figure 1A). In order to give a better visual comparison of the other four species, *Atriplex* was
excluded in the comparison of average shoot biomass (Figure 1B). *Ephedra* developed three times the biomass of *Bromus* and *Pleuraphis*, with *Larrea* being the smallest.

*Atriplex* root systems completely filled their hydroponic tanks. *Bromus* displayed new root growth within 24 hours of transfer to a hydroponic tank with root mass concentrated in the upper half of the nutrient solution. *Ephedra* required several days, after transfer from soil to hydroponic tank, to grow new white roots in contrast to the brown, suberized roots. Root form generally consisted of one long white root reaching to the bottom of the tank with a small mass of shorter roots growing in the upper 10 cm of nutrient solution. *Larrea*’s poor response to the hydroponic environment was evidenced by limited root growth, as several plants developed 10 cm or less total length in new roots following transfer to the hydroponic tanks. *Pleuraphis* displayed new root development 4-5 days after transplant, the more vigorous plants ultimately developing several roots reaching the tank bottom.

In a comparison of the average root biomass for the five species (grown in the hydroponic study), *Atriplex* root biomass exceeded the root biomass of the other species, but the difference was not statistically significant (Figure 2A). *Ephedra* was the next largest at less than one-tenth of the *Atriplex* root biomass and the other three species produced almost the same amount of root biomass (Figure 2B).

A visual assessment that *Atriplex* had substantially more shoot than root biomass was supported by ANOVA analysis (*p* = <0.001, Table 1). In *Larrea*, the mean shoot mass (dry) per plant was equal to the mean root mass (dry) per plant, with *Pleuraphis* and *Bromus* also having their respective shoot mass (dry) equal to their root mass.
(dry). The slightly more shoot biomass than root biomass in *Ephedra* was not statistically significant. A one-way ANOVA found a statistically significant difference between the root/shoot ratios (p = 0.006) and a multiple comparison using Dunn's method clarified the difference was between the ratios of *Atriplex* and *Larrea*, *Bromus* and *Pleuraphis*. *Bromus* had the smallest root-to-shoot ratio, *Larrea* and *Pleuraphis* were slightly larger. *Atriplex* had the largest root to shoot ratio.

As dry weight biomass of the hydroponically-grown plants increased, there was a strong positive correlation with an increasing degree of evapotranspiration (Figure 3, r = 0.82).

Tritium Activity in Leaf Tissue

To understand the factors affecting the amount of tritium activity in leaf tissue, the data were statistically analyzed based on sampling times and levels of tritium treatment. Tritium activity in the leaf tissue of *Atriplex* and the time a sample was collected were found to be significant (p = 0.045) when the data was analyzed using ANOVA. A Tukey test comparing the times that samples were collected did not find any specific differences between the sampling times. No significant difference in tritium activity was found between leaf tissue of plants given the 100 tritium units (T.U.) and leaf tissue of plants given the 1000 T.U. treatment. There was also no significant interaction between the time samples were collected and the level of tritium treatment.
The sampling time was significant ($p = 0.032$) for tritium activity in Larrea leaf tissue, but not for Bromus or Ephedra (Table 2). Tritium treatment was not a significant factor in the measured tritium activity of Bromus or Ephedra.

Whole Plant Transpiration and Tritium Activity in Leaf Tissue

For all species, the tritium activity in leaf tissue (Bq kg$^{-1}$) generally decreased as the volume of whole plant transpiration (grams) increased (Figure 4). On the first day, tritium activity increased to almost 10,000 Bq kg$^{-1}$ before dropping to approximately 1000 Bq kg$^{-1}$ following the mid-morning peak in whole plant transpiration; through the afternoon and next morning decreasing transpiration was associated with increasing tritium activity. On Day 2, the transpiration increased during the morning to slightly above 20 g and remained fairly constant for the rest of the day. Tritium activity ranged from 1000 Bq kg$^{-1}$ to 4000 Bq kg$^{-1}$, until rising above 10,000 B Bq kg$^{-1}$ in late evening. The low tritium activity in the leaves at hour 97 reflected how little tritium was left in the plants due to removal through transpiration. No tritium was detected in the nutrient solution at the end of the 97 hour monitoring period. However, when leaf tritium activity (Bq kg$^{-1}$) and whole plant transpiration (g) were evaluated on a per species basis, the relationships differed from the averaged all-species response depicted in Figure 4. In Atriplex, the variation in whole plant transpiration as measured at each sampling time, was significant ($p = 0.010$, Table 3), whereas tritium treatment was not significant. In Atriplex leaf tissue, tritium activity reached its highest level in the first two samplings (of the monitoring period), then as whole plant transpiration increased, leaf tritium activity decreased.
The peak in whole plant transpiration, in the late afternoon of Day 1, was followed by the lowest tritium activity of the day. There was an overnight increase in leaf tritium activity, which decreased and then began rising as whole plant transpiration increased. The fluctuation in leaf tritium activity on the second day suggests that in *Atriplex*, leaf tritium levels and the quantity of whole plant transpiration was not tightly coupled. The low tritium activity found in *Atriplex* leaf tissue, at the end of the monitoring period, reflected the *Atriplex* transpirational flow that quickly moved the tritiated water through the plant tissue.

In *Bromus*, there was no significant difference in whole plant transpiration from one sampling time to another, nor was tritium treatment significant. On Day 1, fluctuations of tritium activity in leaf tissue (Bq kg$^{-1}$) appeared to operate independently of the whole plant transpiration (g) as the highest tritium activity of approximately 23,000 Bq kg$^{-1}$ occurred in conjunction with the whole plant transpiration high of 15 g. (Figure 5B). On the second day, the whole plant transpiration rose slightly in the morning from the overnight low, dropped to nearly undetectable at midday and exceeded 30 g in the late afternoon before again decreasing to a nearly undetectable level. Overnight, leaf tritium activity dropped from 22,000 Bq kg$^{-1}$ to slightly above 10,000 Bq kg$^{-1}$ in response to rising whole plant transpiration; then increased at midday as transpiration dropped from the Day 2 peak to slightly above a non-detectable level. Lack of a robust data set at some of the sampling times made further conclusions difficult to reach.

In *Ephedra*, no significant variation in whole plant transpiration was found; nor was the level of tritium treatment significant. On Day 1 at the second sampling time,
leaf tritium activity decreased from 12,500 Bq kg⁻¹ as whole plant transpiration rose to a mid-morning high of 15 g, followed by a decrease to a nearly undetectable level in the late afternoon (Figure 5C). Thereafter the tritium activity increased slightly as whole plant transpiration increased. Tritium activity at the final sampling of Day 1 was approximately 13,000 Bq kg⁻¹. Overnight, the leaf tritium activity decreased very slightly. However, by mid-morning of the second day, as the transpiration rate increased, the tissue tritium level decreased slightly, thereafter showing little variation irrespective of transpiration fluctuations.

In *Larrea*, there was no significant variation in quantity of whole plant transpiration during the monitoring period and level of tritium treatment was not significantly correlated with transpiration. There was a significant difference between the tritium activities detected at the various sampling times (p = 0.032). On Day 1, leaf tritium activity and whole plant transpiration increased; with the peak whole plant transpiration occurring overnight (Figure 5D). The highest observed leaf tritium activity occurred at the last sampling of Day 1, and then exhibited an early morning decrease in response to increasing whole plant transpiration. On Day 2, leaf tritium activity again reached a peak in the late afternoon, suggesting a cyclic nature of tritium accumulation and removal in *Larrea* leaf tissue. The simultaneous occurrence of peaks in leaf tritium activity and whole plant transpiration suggests that under the experimental conditions, the transpiration stream was incapable of moving the tritiated solution through the plant with sufficient rapidity to prevent elevated tritium accumulation. It also suggests the mechanisms operating in the other species that
established an association between increasing transpiration and decreasing leaf tritium did not function in the same way in \textit{Larrea}.

Tritium Activity in Leaf Transpiration by Species

A goal of this project was to identify which of the species, grown in the study, would be the best sentinel of tritium contamination. When all the species data was analyzed (ANOVA) in the same tests, no significant differences were found for tritium activity in transpiration samples collected from \textit{Atriplex}, \textit{Ephedra}, \textit{Larrea} and \textit{Pleuraphis}. No significant difference was found between the 100 T.U. and 1000 T.U. treatments.

In \textit{Atriplex}, no statistically significant interaction was found between sampling time and the level of tritium treatment. On the first day of sampling \textit{Atriplex}, an increase in whole plant transpiration was paralleled by a delayed response in the tritium level in transpirational capture. Overnight, the transpiration tritium activity decreased, its lowest activity detected in the early morning sampling (taken on the second day) (Figure 6A). On the second day, the transpiration tritium activity appeared to more closely follow the fluctuations in whole plant transpiration than on the first day.

When the \textit{Bromus} data was analyzed separately, sampling time significantly influenced the tritium activity found in transpiration samples ($p = 0.014$, Table 4); however no significant influence of tritium treatment was observed. Transpiration tritium levels were somewhat in phase with changes in whole plant transpiration during the first thirty hours following tritium addition to the hydroponic tanks; increases in whole plant transpiration were followed by decreases in transpiration.
tritium activity (Figure 6B). There was an association between whole plant transpiration and tritium activity in transpiration in the morning on the second day, but not in the afternoon; the highest observed transpiration tritium activity in *Bromus* was found at the same sampling as the second day peak in whole plant transpiration.

In *Ephedra*, there was an initial parallel increase in transpiration tritium activity as whole plant transpiration increased on Day 1 (Figure 6C). Tritium activity in transpiration decreased overnight and, on Day 2, increased at midday in a delayed response to whole plant transpiration rates that rose slightly from the overnight low. On the second day, there was less association between transpiration tritium activity and whole plant transpiration rate than on the first day.

In *Larrea*, the whole plant transpiration rate gradually increased to a peak value on the morning of the second day (Figure 6D). The tritium activity in transpirational capture was highest on the first day of monitoring and decreased to its lowest value overnight. The activity of tritium remained low on the second day until the final sampling time. The transpiration tritium activity did not show any association with the whole plant transpiration rate.

The lack of a robust data set made it difficult to establish any association between whole plant transpiration rates and transpiration tritium activity in *Pleuraphis* (Figure 6E). Problems with extreme lumex values were encountered when counting tritium activity in *Pleuraphis* samples.
Correlating Tritium Activity in Leaf Tissue and Transpiration

Accumulation of tritium activity in leaf tissue is related to the speed with which transpiration removes tritiated water vapor from leaf tissue. A significant species effect was found for the ratio of transpiration tritium (Bq L⁻¹) to tissue tritium (Bq kg⁻¹) (p = 0.008), but the effect of the level of tritium treatment was not found to be significant. An accompanying Tukey test attempted to isolate the difference between the species, and in the process, *Atriplex* was found not to be significantly different from *Bromus* and all other species-to-species comparisons were classified as ‘do not test’. The discrepancy was related to the data distribution failing to pass the normality test, or to the large difference between the *Atriplex* ratio of transpiration tritium (Bq L⁻¹) to tissue tritium (Bq kg⁻¹) and the ratios of the other species. *Atriplex* had ratios greater than 40.0 in contrast to ratios of 2.0 or less for the other species. A second ANOVA found sampling time and level of tritium treatment had no significant effect on the ratio of transpiration tritium (Bq L⁻¹) to tissue tritium (Bq kg⁻¹). Finally, a third test based on sampling time and species found only species was significant for the ratio of transpiration tritium (Bq L⁻¹) to tissue tritium (Bq kg⁻¹) (p = 0.039).

On the basis of other evidence suggesting *Atriplex* might be driving the species difference discussed above, ANOVAs were performed with *Atriplex* removed from the data set. The first test found species and tritium treatment had no significant effect on the transpiration tritium (Bq L⁻¹) to tissue tritium (Bq kg⁻¹) ratio. The second test found sampling time and tritium treatment had no significant effect on the tritium ratio. Finally, a third test was performed solely on *Atriplex*, finding that sampling time and tritium treatment had no significant effect on the tritium ratio.
Hourly comparisons of tritium activity in tissue (Bq kg\(^{-1}\)) relative to transpiration tritium activity (Bq L\(^{-1}\)) are plotted in Figures 7, 8A and 8B. At hour four of the monitoring period, tritium activity was higher in leaf tissue, whereas by hour nine, the tritium activity was greater in the transpiration samples, suggesting that the tritium may have been moving out of the plant in the transpirational stream (Figure 7 and 8A). At later sampling times, there was no apparent correlation between tritium activity in leaf tissue or in transpiration as there was great variation in the values. Overall, tritium activity for the entire 97 hour monitoring period displayed a weak non-significant trend of increasing tritium activity in leaf tissue associated with decreasing tritium activity in transpiration (Figure 7). On the first day of monitoring, there was a clustering of low tritium activity in both tissue and transpiration (Figure 8A). On the second day, there was a greater spread in tritium activity in the transpiration than in the leaf tissue (Figure 8B). The most noticeable difference between Atriplex and the other species was that most of the values lowest in tissue tritium activity were associated with Atriplex (Figure 8C).

Correlating Tritium Activity in Transpiration and Biomass

Possible correlations (ANOVA) between tritium activity in transpiration (Bq L\(^{-1}\)) and biomass (g) were assessed. There was no significant difference in the transpiration tritium activity based on the activity of tritium treatment or the quantity of biomass. A significant difference between species was found when the ratio of tritium activity in transpiration (Bq L\(^{-1}\)) and the quantity of biomass (g) was analyzed based on species and sampling times (p =<0.001). Significant differences in these
ratios was observed between Larrea and all other species (p < 0.001), between Bromus and Atriplex (p = 0.002), and between Bromus and Ephedra (p = 0.008). A significant difference based on sampling times was observed only between the sampling performed at Hour 37.5 and the sampling performed at Hour 31 (p = 0.032). ANOVAs were conducted for each species, comparing sampling time and tritium treatment with a ratio of transpiration (Bq L⁻¹) to biomass (g). In Bromus, there was no significant difference in the ratio based on sampling times; but a significant difference was observed based on tritium treatment (p = 0.008). There were no significant differences in the ratio based on sampling times or tritium treatments in the other species.

The effects of species and tritium treatment interactions on the relationship between tritium levels in transpiration (Bq L⁻¹) and quantity of biomass (g) were investigated. A significant difference was found between species (p =<0.001); specifically, between Larrea and Atriplex (p < 0.001), Larrea and Ephedra (p = 0.001), Bromus and Atriplex (p < 0.001), and between Bromus and Ephedra (p < 0.001). Although, there was not a significant difference between levels of tritium treatment, an interaction between species and the level of tritium treatment (p = <0.001) was found. Based on species, a comparison of transpiration (Bq L⁻¹): biomass (g) found a significant difference in the way Larrea responded to the 100 T.U. treatment (p < 0.001). Comparison of that same ratio, based on species, found significant differences between Bromus and Atriplex (p < 0.001), Ephedra (p < 0.001), Larrea (p = 0.040) and Pleuraphis (p = 0.008) for the 1000 T.U. treatment.
The effects of sampling times and tritium treatment on the relationship between tritium levels in transpiration (Bq L⁻¹) and quantity of biomass (g) were analyzed. However, no significant difference in the ratio was observed based on the sampling times or tritium treatments.

In this experiment, tritium was applied in a single pulse, therefore if the quantity of biomass (g) produced by a plant was instrumental in determining the rate at which the plant purged itself of tritium activity, *Atriplex* had a potential advantage. There was a significant difference between the biomass of *Atriplex* and biomass of the other species (p = <0.001). Biomass did not influence any interaction between sampling time and tritium treatment. To further explore the differences found for *Atriplex*, a comparison (ANOVA) between sampling times and biomass, based on transpiration tritium (Bq L⁻¹), was performed for only *Atriplex* samples. No significant difference in transpiration tritium activity was found, based on sampling times or biomass.

When the transpiration tritium activity for all transpiration samples was plotted against the total biomass collected during the monitoring period, it was interesting to note that the data spread was predominately along the y-axis representing biomasses of less than 10 g. In those low weight samples, the range of transpiration tritium activity spanned from nearly undetectable to over 30,000 Bq L⁻¹. The low biomass reflects the small size of most plants in the study and the range of tritium activity indicated that a simple relationship between low biomass and tritium activity does not exist for all the species in this study (Figure 9). The biomass values above 20 g also show a variation in tritium activity, with clustering below 20,000 Bq L⁻¹. In *Atriplex* a strong positive correlation (Figure 10A, r = 0.73) existed between increasing biomass...
and increasing transpirational tritium activity measured during the experiment. In *Bromus, Ephedra, Larrea* and *Pleuraphis*, low biomass was not tightly associated with any particular level of transpirational tritium activity as each species revealed considerable variation (Figure 10B).

The daily transpiration tritium activity (Bq L⁻¹), as related to biomass, is shown in Figures 11A through 11E. At hour four of the monitoring period, the low biomass plants had tritium activities generally less than 10,000 Bq L⁻¹, although one sample was above 30,000 Bq L⁻¹ (Figure 11A). The larger biomass plants gave so few reliable transpirational samples at hour four; no conclusions can be derived from the figure about a relationship between transpiration tritium activity and the biomass of these plants. At hour six, the tritium activity was more widely spread in the low biomass plants and an association between increasing biomass and decreasing tritium activity was developing (Figure 11B). At hour nine, most samples from low biomass plants were above 10,000 Bq L⁻¹; however, in plants greater than 40 g in biomass (represented exclusively by *Atriplex*) the tritium activity was 10,000 Bq L⁻¹ or less (Figure 11C). At hour 10.5, the tritium activity of samples from large biomass plants increased slightly. Two samples were above 10,000 Bq L⁻¹ and one was above 30,000 Bq L⁻¹ at hour 10.5 (Figure 11D). In the final sampling on the first day of monitoring, tritium activity in the low biomass samples ranged from almost no tritium activity to approximately 25,000 Bq L⁻¹ (Figure 11E). In the larger biomass samples, the tritium activity was at or above 10,000 Bq/L, with the highest measured activity above 30,000 Bq L⁻¹.
At hour 26 (first sampling on the second day during the monitoring period), the majority of samples came from low biomass plants and tritium activity was clustered around 5,000 Bq L\(^{-1}\) or less (Figure 12A). Low biomass samples collected at hour 28 had a wider variation in tritium activity, ranging from nearly undetectable to 25,000 Bq L\(^{-1}\) (Figure 12B). Also, a greater number of large biomass plants had measurable tritium activity than at the previous sampling, revealing an association between high biomass (g) and low tritium activity in transpiration (Bq L\(^{-1}\)). At hour 31.5, the tritium activity in the low biomass plants was similar to that seen in the previous sampling (Figure 12D). In the plants with a biomass greater than 40 g, some tritium activity above 10,000 Bq L\(^{-1}\) was observed. At hour 33.5 of the monitoring period, the tritium activity in low biomass plants generally clustered at 8,000 Bq L\(^{-1}\) or less (Figure 12D). Increased tritium activity was found in several samples collected from large biomass plants (Atriplex, Figure 12E). The results found at hour 35.5 were similar to the previous sampling at hour 33.5 (Figure 12F). In the final sampling taken at hour 97, low levels of tritium activity were measured at all biomasses, reflecting depletion of tritium activity in the nutrient solutions (Figure 12G). Samples of nutrient solution were collected from each tank at the end of the monitoring period and counted; in the majority of the samples, tritium activity was below the detection level.

Correlating Tritium Activity in Leaf Tissue and Biomass

Comparison of the highest tritium activity measured in tissue samples (collected during the hydroponic study), revealed a curvilinear association between low biomass and higher tissue tritium activity (Figure 13, \(r = 0.61\)). The highest tritium activity
was found in plants with a total dry biomass of 2 g or less. A comparison of all levels of tritium activity (Figure 14) indicated that low biomass plants had the highest tritium activities, just below 20,000 Bq kg$^{-1}$ in the leaf tissue, whereas plants 40 g or greater exceeded tritium activities of 10,000 Bq kg$^{-1}$ in only two samples.

To determine the impact of sampling times, tritium treatment, and species, on the relationship between tritium activity in leaf tissue (Bq kg$^{-1}$) and biomass (g) that was depicted in the figures listed above, several two-way ANOVAs were performed. A ratio of tissue tritium activity (Bq kg$^{-1}$) to biomass (g) was entered as the dependent variable. Sampling times were found to be marginally significant ($p = 0.055$). A significant difference was found in the ratio response of each species to sampling times ($p = < 0.001$), and to level of tritium treatment ($p = 0.0001$). The differential response to sampling times was found between *Pleuraphis* and *Ephedra* ($p = 0.007$), *Pleuraphis* and *Atriplex* ($p = 0.006$), *Larrea* and *Ephedra* ($p < 0.001$), *Larrea* and *Atriplex* ($p < 0.001$), *Bromus* and *Ephedra* ($p = 0.016$), and between *Bromus* and *Atriplex* ($p = 0.007$). There was a significant interaction between species and level of tritium treatment ($p = 0.001$). Tukey tests identified that *Larrea* ($p < 0.001$) and *Pleuraphis* ($p = 0.018$) responded to the 100 T.U. treatment differently than to the 1000 T.U. treatment. Comparison of tritium activity per unit of biomass found samples from 100 TU *Pleuraphis* averaged greater than 3 times the tritium activity found in samples from 1000 TU *Pleuraphis*. In *Larrea*, the 100 TU samples averaged 2.25 more tritium activity per sample than the 1000 TU samples. *Pleuraphis* and *Larrea* did not differ from each other in the amount of tritium activity generated by the 100 T.U. treatment per biomass unit of leaf tissue, but they did vary from the other
three species (p < 0.001). *Bromus* and *Larrea* responded similarly to the 1000 T.U. treatment and differed from the other 3 species. More specifically, *Bromus* significantly differed from *Atriplex* (p = 0.002) and *Ephedra* (p = 0.014) while *Larrea* significantly varied from *Atriplex* (p = 0.006) and slightly varied from *Ephedra* (p = 0.041).

The statistical tests (described in the previous paragraph) did not identify *Atriplex* as differing from the other species. On the basis of *Atriplex*’s large biomass being significantly linked to other differences, an analysis was conducted on *Atriplex* data to determine what impact sampling time or tritium treatment had on the ratio of tissue tritium activity (Bq kg⁻¹): biomass (g). A significant difference in the ratio was found based on sampling times (p = 0.048), but tritium treatments were not found to be significant, with no significant interaction between sampling time and tritium treatment on the ratio.

Following the addition of tritium to the hydroponic tanks, tissue samples from low biomass plants had tritium activities either below 6,000 Bq kg⁻¹ or above 16,000 Bq kg⁻¹. The larger biomass plants had less than 2000 Bq kg⁻¹ in their tissue, with a linear correlation between high biomass and low tritium activity (Figure 15A, r = 0.71, p = 0.039) for all samples collected at hour four. This is in contrast to the previously reported r-value of 0.81 for samples of the highest tritium activity measured in tissue samples collected during the hydroponic study (Figure 13). The association between tritium activity and biomass changed at the second sampling time of the first day of monitoring with plants of 150 g biomass or greater showing tritium activities as high as 40,000 Bq kg⁻¹ while plants of lower biomass typically had tritium activities of
less than 10,000 Bq kg\(^{-1}\) (Figure 15B). The early afternoon sampling at hour 9 on the 
first day again found a correlation between large biomass and low tissue tritium 
activity (Figure 15C, \(r = 0.85, p < 0.001\)). Plants with low biomass had 14,000 Bq kg 
\(^{-1}\) or less in tissue tritium activity. By 12.5 hours, there was a relationship between low 
biomass and high tritium activity in leaf tissue or the converse, high biomass and low 
tissue tritium activity (Figure 15E, \(r = 0.66, p = 0.04\)).

At the early morning sampling on the second day of tritium monitoring, low 
biomass plants had tissue tritium activity ranging from approximately 2000 Bq kg\(^{-1}\) to 
almost 40,000 Bq kg\(^{-1}\) (Figure 16A). Plants with greater than 50 g biomass had 
tritium activities of 2,000 Bq kg\(^{-1}\) or less in their tissue. The most reliable samples for 
the hour 28 sampling came from the larger plants, with the data suggesting an 
association between high biomass and low tissue tritium activity was being maintained 
(Figure 16B). By 31 hours, tritium activities between 8,000 and 10,000 Bq kg\(^{-1}\) were 
found in the low biomass samples, while the high biomass samples were characterized 
by tritium activities at or below 2,000 Bq kg\(^{-1}\) (Figure 16C, \(r = 0.85, p = 0.005\)). The 
correlation between high biomass and low tissue tritium activity was continued in the 
hour 33 sampling (Figure 16D, \(r = 0.88\)). Low biomass plants had tissue tritium 
activity clustering between 15,000 Bq kg\(^{-1}\) and 30,000 Bq kg\(^{-1}\). Due to the small size 
of most plants grown in the hydroponic study and the need to have samples available 
for collection at later sampling times, all data obtained at hour 35.5 were collected 
from \(Atriplex\). Thus all the plants sampled at hour 35.5 had biomass greater than 20 g 
with tissue tritium activity of less than 2,000 Bq kg\(^{-1}\) (Figure 16E). The final 
sampling on the second day of monitoring was consistent with results obtained from
the final sampling on day one (Figure 16F). Low biomass plants ranged from
approximately 1000 Bq kg\(^{-1}\) to 36,000 Bq kg\(^{-1}\) tissue tritium activity. Plants with a
biomass greater than 20 g had tissue tritium activities of less than 1000 Bq kg\(^{-1}\).
Samples taken during the final collection of the monitoring period at hour 97 had
tissue tritium activities of 8,000 Bq kg\(^{-1}\) or less in low biomass plants and tritium
activities of less than 1000 Bq kg\(^{-1}\) in high biomass plants (Figure 16G).

Tritium Partitioning in the Plant

Root samples were taken on the final day of monitoring. Root tritium activities
were then correlated with leaf tritium activities (from samples taken on the final day of
monitoring). A one-way ANOVA between the tritium activity in root tissues and the
tritium activity in leaf tissues, indicated significant differences (p = 0.004). No
significant difference was found between the five species for the amount of residual
tritium activity in root tissue. The range in the leaf tissue varied from no residual
tritium activity to 40,000 Bq kg\(^{-1}\). Four root samples still contained high tritium
activity. The root tritium activity in these four samples was approximately 25,000 Bq
kg\(^{-1}\) with a corresponding leaf tritium activity of less than 10,000 Bq kg\(^{-1}\).

No significant difference was found between the five species when the ratio of root
tritium activity/shoot (leaf) tritium activity was compared. The variability of tritium
activity found in a comparison of root to shoot tritium activities was too high to make
conclusive statements about the results, although the comparison did reveal that
Ephedra had the largest ratio, approximately 6 times greater than the second largest
ratio (that of Atriplex, Figure 17). The Bromus ratio was approximately one-fifth of
that measured for \textit{Atriplex}, with \textit{Larrea} and \textit{Pleuraphis} having root to shoot tritium activities less than \textit{Bromus}. The small root to shoot tritium activity ratio of \textit{Larrea} reflected the large amount of tritium being held in the leaf tissue in association with low transpiration estimates.

\section*{Column Study}

\textbf{Growth Response of Above Ground Biomass}

A significant difference in above ground biomass was found between the three species grown in the column study ($p = < 0.001$). The difference was identified as being between \textit{Tamarix} and \textit{Larrea} by Dunn's method. \textit{Tamarix} produced approximately 1.4 times more aboveground biomass than \textit{Atriplex}, and 12 times more than \textit{Larrea} (Table 1). The six \textit{Tamarix} plants were already year old seedlings with well-developed root systems extending the full length of the columns, when brought into the greenhouse for this study. The six \textit{Larrea} seedlings were transplanted from 10-cm starter pots, with small root systems. The six \textit{Atriplex} were also transplanted from 10-cm starter pots, but the root systems had filled the pots completely and were beginning to grow out through the drainage holes. \textit{Larrea} had better growth in the columns than in hydroponic tanks, but the inherent life-history traits that lead to slower maturation in this species than in \textit{Atriplex} resulted in \textit{Larrea} producing less biomass than \textit{Atriplex}. 

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Distribution of Tritium in Soil

Following the harvest of all aboveground biomass, the PVC columns were sectioned to take soil and root samples. After application of a decay correction factor and an efficiency correction factor, the effective tritium activity remaining in the soil 173 hours after input was non-detectable using our experimental approach.

Soil samples, taken during installation of the input ports, were analyzed for gravimetric water content (g water/g soil). There was no significant difference in the soil gravimetric water content in the columns based on species. However, soil in the Atriplex columns had the highest average gravimetric water contents and Tamarix had the lowest average gravimetric water contents (Figure 18). Samples for gravimetric analysis were not taken at the end of the column study because the processing of such samples could have potentially contaminated equipment being used for other ongoing, non-tritium studies.

Because little, if any, tritium was detected in the soil, tritium activity in the root tissue was chosen as the indicator of tritium having reached a particular depth in the soil. Sampling points below the input ports were chosen based on the lowest observed level of root biomass in the clear plexiglass columns. In Atriplex, no significant difference existed between the sampling positions or between the levels of tritium treatment. However, samples taken from the bottom of the columns had the highest tritium activity, roughly twice the activity found at 17 cm above and 17 cm below the input port (Figure 19). The roots appeared to have translocated the tritiated solution downwards in the root tissue, in addition to tritium moving up the root system via transpirational flow.
In *Larrea*, there was a significant difference between the tritium activities found in root tissue ($p = 0.007$) collected at the four sampling points that were established during the post-study sectioning of each column. The difference was identified as between the sampling point below the input port (below) and the sampling point above the input port (above, $p = 0.028$); and between the sampling point below the input port (below) and the sampling point at the end of the column (end, $p = 0.017$). The level of tritium treatment did not influence the location of tritium activity. The largest (and approximately equal) tritium activities were found in samples from the input port and 17 cm below the port, the smallest activity was found 17 cm above the port and at the end of the columns (Figure 20). The low tritium activity at the end of a column suggests that the tritiated solution did not move the entire length of the column associated with internal drainage. The low activity found 17 cm above the input port suggests the upper regions of the root system were cleared of most tritium by upward and downward translocation.

Based on the level of tritium activity in the root tissue of *Tamarix*, there was no significant difference between the tritium treatments imposed or sampling position in the columns. However, the highest tritium activities in root tissue were measured at the port sampling position, at approximately eight times greater than the tritium activity found at 17 cm below the input port (Figure 21). The insignificance of these results renders interpretation difficult and suggests further experimentation to verify if the root mechanisms operating in *Tamarix* are the same as those operating in the other species.
Tritium Activity in Leaf Tissue by Species

To identify species commonalities and differences in response to the tritium treatments, several two-way ANOVAs were performed. Sampling time and tritium treatments did not have a significant influence on the tritium activity found in leaf tissue; also there was no significant interaction between sampling time and tritium treatment. When species and sampling time were compared on the basis of tritium activity in leaf tissue, *Larrea* significantly differed from *Atriplex* and *Tamarix* (p = < 0.001) with the differences being between hour 2 and hours 10, 28, 32, and 173 (all p < 0.01). Significant differences were found between hour 26 and hours 10, 28, 32, and 173 (all p < 0.05). *Larrea* differed significantly from *Atriplex* at hours 2, 5, 7, 26, and 34 (all p < 0.05). *Larrea* significantly differed from *Tamarix* at hours 2, 5, 7, 26, and 34 (all p < 0.001).

There were no significant differences between the tritium activity in *Atriplex* leaf tissue based on tritium treatment or sample time collection. There was also no significant interaction between these factors. Tritium activities in *Atriplex* leaf tissue were averaged for each sampling time of the 173-hour monitoring period (Figure 22A). Peak tritium activity on the first day occurred mid-day reaching 15,000 Bq kg$^{-1}$ and then dropping to approximately 4,000 Bq kg$^{-1}$. On day 2 of the monitoring period (the first sampling), there was a slight increase in tritium activity above the previous evening's low point. At midday, the tritium activity in leaf tissue had dropped to the lowest level of the monitoring period, followed by a late afternoon increase to a level slightly higher than the morning activity level. At the final sampling (hour 173), a slight decline in tritium activity was observed.
The importance of sampling time for tritium activity in *Larrea* leaf tissue was confirmed (ANOVA, p = 0.016), however a Tukey test did not identify which hours were significantly different. No significant difference was found between the tritium treatments after allowing for differences in sampling times. No significant interaction between sampling time and tritium treatment was found. A significant difference was found between the 1,000 T.U. treatment and the 100 T.U. treatment at hour 34 of the monitoring period (p = 0.032). The highest tritium activities were above 25,000 Bq kg$^{-1}$ in *Larrea* samples taken at the first sampling time of each day (Figure 22B). Over the first day of monitoring, the tritium decreased to its lowest activity at the evening sampling (10,000 Bq kg$^{-1}$). Tritium activity on the second day varied from the first day by reaching the lowest activity (slightly less than 10,000 Bq kg$^{-1}$) at the mid-morning sampling, then increasing to approximately 24,000 Bq kg$^{-1}$ by the final sampling of the day. On the final day of monitoring, tritium activity was slightly above 10,000 Bq kg$^{-1}$.

For *Tamarix*, neither sampling time nor the level of tritium treatment had a significant effect on the tritium activity in leaf tissue. There was also no significant interaction between sampling time and level of tritium treatment on leaf tissue tritium activity. Tritium activity for *Tamarix* on the entire first day of monitoring varied by less than 2,000 Bq kg$^{-1}$ from the initial activity of slightly above 4,000 Bq kg$^{-1}$, with the daily low activities occurring at the mid-afternoon sampling (Figure 22C, r = 0.37, p = 0.01). The highest activity on day 1 of the monitoring period occurred during the final sampling of the day. Tritium activity, at the first sampling on day 2 of the monitoring period, was less than 1,000 Bq kg$^{-1}$ lower than the final activity on day 1.
The peak tritium activity found in *Tamarix* (during the monitoring period) occurred mid-afternoon on Day 2, reaching approximately 10,000 Bq kg\(^{-1}\). On the final day of monitoring (at hour 173), the tritium activity was approximately 7,000 Bq kg\(^{-1}\). Interestingly, only *Tamarix* revealed an increasing trend of tritium activity in leaf tissue by the end of the experiment, while tritium activity declined in *Atriplex* and *Larrea*. This distinction in tritium activity may be related to another distinction between *Tamarix* and the other species; the roots of the *Tamarix* plants were highly suberized in the unsaturated zone where the tritium was introduced.

Temporal Response of Tritium in Leaf Transpiration by Species

The three species in the column study were compared based on tritium activity in leaf transpiration (Bq L\(^{-1}\)). An ANOVA comparing the species and times of sample collection found that tritium activity in *Larrea* leaf transpiration significantly differed from *Tamarix* (\(p < 0.001\)) and *Atriplex* (\(p = 0.003\)), while *Atriplex* and *Tamarix* exhibited similar responses. Sampling time was not found to have a significant effect on tritium in leaf transpiration. Another test found a significant difference in species response to the two tritium treatments (\(p = < 0.001\)); *Larrea* responded to the 100 T.U. treatment differently than *Tamarix* (\(p = 0.005\)) or *Atriplex* (\(p = 0.025\)). The mean tritium activity for *Larrea* in the 100 TU treatment was 24,521 Bq L\(^{-1}\) compared to 11,846 Bq L\(^{-1}\) for *Atriplex* and 9,099 Bq L\(^{-1}\) for *Tamarix*. No similar distinction was found in response to the 1000 T.U. treatment, although the difference between *Larrea* and *Atriplex* was marginally significant (\(p = 0.055\)). In contrast, there was a marginally significant difference between the two tritium treatments (\(p = 0.078\) after
allowing for the differences in the species. Also, the lack of significant interaction between species and tritium treatment suggests the variation in species response was not dependent on the activity of tritium treatment.

In Atriplex, neither sampling time nor level of tritium treatment was significant. The highest average tritium activity in transpiration was approximately 23,000 Bq L$^{-1}$ which was detected at the initial sampling of the monitoring period (Figure 23A), followed by decreasing tritium activity associated with the midday and mid-afternoon samplings. Although, the lowest level of tritium activity detected during the first two days of monitoring occurred during the afternoon of Day 1, by early evening the tritium activity in the transpiration samples increased to approximately 15,000 Bq L$^{-1}$. The first sampling (of the second monitoring day) had the lowest tritium activity of the day (~4,000 Bq L$^{-1}$) with the next two samplings showing increasing activity, eventually reaching 10,000 Bq L$^{-1}$. The final sampling (at hour 173) detected almost no tritium activity.

In Larrea, there was no significant difference between sampling times or level of tritium treatments. The transpiration data for Larrea was less robust than the data for Atriplex or Tamarix, therefore the data was plotted (Figure 23B) for the initial 34 hours of monitoring. In the hydroponic study and in the column study, tritium activity in Larrea transpiration appeared to be cyclic in nature. From the initial mid-morning sampling to the mid-day sampling, tritium activity declined. The mid-afternoon sampling detected that the highest activity of the day was greater than 30,000 Bq L$^{-1}$. On Day 2, tritium activity in the transpiration declined, with the lowest activity of approximately 8,500 Bq L$^{-1}$ detected at the first sampling of that day. The highest
tritium activity of the entire monitoring period occurred on the second day, reaching over 30,000 Bq L\(^{-1}\) at hour 28.

In *Tamarix*, sampling time had a significant impact on transpiration tritium activity (\(p = 0.002\)); hour 32 differed from hours 2, 5, 7, 10, 34, 173 (all \(p < 0.033\)). No significant difference in transpiration tritium activities was found based on tritium treatments. Tritium activity in transpiration samples remained below 10,000 Bq L\(^{-1}\) on the first day of monitoring (Figure 23C). On the second day, the initial tritium activity was approximately 11,000 Bq L\(^{-1}\), increasing to the highest sampled activity of approximately 28,000 Bq L\(^{-1}\) at hour 32. The final sampling found a nearly undetectable tritium activity.

Correlating Leaf and Transpiration Tritium Activities

ANOVAs were performed to examine the impact of transpiration tritium activity on leaf tritium activity. No significant difference based on species was found between tritium activity in leaf tissue and in transpiration. There was a significant difference in species response to the time when samples were collected (\(p = 0.022\)), based on a ratio of the tritium activity in leaf tissue (Bq/kg) to the tritium activity in transpiration (Bq L\(^{-1}\)). *Atriplex* differed significantly in this tritium ratio from *Tamarix* (\(p = 0.043\)), but not from *Larrea*. A comparison based on the tritium treatments and the sampling times indicated that both treatment activity and sampling time were not significant, nor was there a significant interaction between the species used in the column study and the activity of tritium treatment. One-way ANOVAs based on each species,
confirmed the tritium activity in transpiration (Bq L \(^{-1}\)) was not significantly different from the tritium activity in leaf tissue.

A plot of transpiration tritium (Bq L \(^{-1}\)) against leaf tissue tritium (Bq kg \(^{-1}\)) for all species, indicated that the tritium activity in a transpiration sample was sometimes higher than the activity in the corresponding leaf tissue sample, some corresponding samples appeared to be in equilibrium and sometimes transpiration tritium activity was less than leaf tritium activity (Figure 24A). Separating the samples on a species basis suggested that the variation in Figure 28 was not a species effect. When Atriplex, Larrea and Tamarix activities in transpiration and leaf tissue were plotted separately (Figure 24 B, C and D), no statistical correlations could be established.

Correlating Biomass and Tritium Activities in the Plants

To determine if biomass might be influencing the degree of tritium activity in transpiration (Bq L \(^{-1}\)) that was detected in each species, an ANOVA was conducted, comparing the three species and the two tritium treatments on the basis of biomass. Biomass of Tamarix and Larrea were found to differ significantly (p < 0.001) as were Tamarix and Atriplex (p = 0.001) and Atriplex and Larrea (p < 0.001). The difference was not due to the activity of tritium treatment; there was no significant interaction between species and level of tritium treatment. Using a ratio of transpiration (Bq L \(^{-1}\)) to biomass (g), each species was compared based the sampling times; there was a significant difference between Atriplex and Tamarix (p < 0.001) and between Larrea and Atriplex (p < 0.001). The time of sample collection, however, was not found to have a significant effect on the tritium transpiration to biomass ratio.
A second test using the same ratio comparing species and tritium treatments found a significant difference between *Larrea* and the other species (*p < 0.001*) and between the activity of tritium treatment (*p = 0.003*). In *Larrea*, there was a significant difference in response to the levels of tritium treatment (Tukey test, *p < 0.001*). In the 1000 T.U. treatment, significant interaction between species and tritium treatment was found for *Larrea* (*p = < 0.001*).

The highest tritium activities detected in *Larrea* transpiration samples were easily distinguished from the highest tritium activities found in *Atriplex* and *Tamarix* (Figure 25A). Tritium activities above 30,000 Bq L⁻¹ associated with the low biomass combined to segregate the *Larrea* data points in the upper left quadrant of the graph while the tritium activities of *Atriplex* and *Tamarix* occupied similar positions in the center of the figure, with tritium activities ranging from less than 10,000 Bq L⁻¹ upwards to 40,000 Bq L⁻¹. When the transpiration tritium activity for all samples was plotted against biomass (Figure 25B), *Larrea* remained separate because of low biomass values; the majority of *Atriplex* and *Tamarix* were clustered midway along the biomass axis. Some *Tamarix* were distinguished primarily by biomass rather than tritium activity, suggesting the low tritium activity per unit of biomass may have resulted as an effect of tissue dilution.

In hour 2 of the monitoring period (Figure 26A), no distinctive association between tritium activity in transpiration and biomass was apparent as tritium activity was generally below 10,000 Bq L⁻¹ and biomass ranged from approximately 2 g to above 40 g. The majority of the transpiration samples collected at hour 5 were associated with biomasses of 10 g to 30 g, tritium activity ranged from slightly detectable to
almost 25,000 Bq L\(^{-1}\) (Figure 26B). By hour 7, a significant curvilinear correlation began to develop between decreasing tritium activity in transpiration and increasing biomass (Figure 26C, \(r = 0.86, p = 0.03\)). A linear correlation was beginning to develop by hour 10, although not statistically significant (Figure 26D, \(r = 0.49, p = 0.126\)). By hour 28 (on the second day of monitoring), a linear correlation developed between increasing tritium activity and decreasing biomass was again revealed to be linear (Figure 26E, \(r = 0.68, p = 0.0188\)). The only samples collected at hour 32 were *Atriplex*, with five samples clustered between 15-24 g biomass and 3,000-25,000 Bq L\(^{-1}\) tritium activity, with a sixth sample having a biomass greater than 40 g biomass associated with a tritium activity of 35,000 Bq L\(^{-1}\) tritium activity (Figure 26F). At the final sampling of the monitoring period (hour 173), there was no significant correlation between low tritium activity and high biomass (Figure 26G, \(r = 0.82, p = 0.1355, n = 17\)).

The relationship between tritium activity in leaf tissue (Bq kg\(^{-1}\)) and biomass (g) were also examined (ANOVA). Sampling times and levels of tritium treatment were not significant and there was no significant interaction between them. Species was a significant factor: *Tamarix* differed from *Larrea*, *Tamarix* differed from *Atriplex*, and *Atriplex* differed from *Larrea* (all \(p < 0.001\)).

To explore the concept that the quantity of biomass produced by a plant could affect the accumulation of tritium, a ratio of the tritium activity in leaf tissue (Bq kg\(^{-1}\)) to biomass (g) was analyzed (ANOVA). The first test compared the three species and the sampling times. The ratio for *Larrea* was found to differ significantly from *Tamarix* (\(p < 0.001\)) and *Atriplex* (\(p < 0.001\)) at two specific times in the monitoring period,
hour 7 (Tamarix p = 0.023, Atriplex p = 0.035) and hour 34 (Tamarix p < 0.001, Atriplex p < 0.001). Using the same ratio, the second test compared the sampling times to the activity of tritium treatments. Although the time of sampling was not significant, there was a significant relationship between sampling time and level of tritium treatment (p = 0.002), particularly at hour 7 (p = 0.036) and hour 34 (p = 0.027). Again using the ratio of leaf tritium/biomass, comparison of the three species indicated differences between the way Larrea and the other species (Tamarix p < 0.001, Atriplex p < 0.001) responded to the activity of tritium treatments. The level of tritium treatment applied to a column was significant (p = 0.001) and a Tukey test comparing the 1,000 TU treatment to the 100 TU treatment identified Larrea as responding more strongly to the 1,000 T.U. treatment (p <0.001, difference of mean = 15,227.636). Comparisons between the leaf tritium/biomass responses of the three species to the 1,000 T.U. treatment indicated Larrea differed from the other two species (Tamarix p < 0.001, Atriplex p < 0.001) with a 200 times greater difference in the mean (Tukey test).

Comparing the highest tritium activity found in leaf tissue samples to the total biomass of the respective plant indicated Larrea distinctly segregated from the other species (Figure 27A), very similar to the segregation observed when tritium activity in transpiration was compared to biomass (Figure 25A, r = 0.70, p = 0.001). Larrea was clustered in the region of tritium activity above 20,000 Bq kg$^{-1}$ with less than 10 g of biomass. Biomass and tritium in the leaf tissue of Atriplex and Tamarix were clustered together with biomass between 15-25 g and tritium in the leaf tissue primarily below 20,000 Bq kg$^{-1}$. When leaf tritium activity was plotted against the
total shoot biomass (Figure 27B, \( r = 0.59, p <0.001 \)), *Larrea* clustered to the left side of the graph (below 5 g in biomass) and with tritium activities ranging from slightly above zero to almost 40,000 Bq kg\(^{-1}\). All of the *Atriplex* and most of the *Tamarix* were centered on the graph between 10-25 g biomass and primarily below 20,000 Bq kg\(^{-1}\) of tritium in the leaf tissue. In addition, several *Tamarix* samples were clustered at greater than 40 g in biomass and below 10,000 Bq kg\(^{-1}\), suggesting a correlation between decreasing tritium activity in leaf tissue as the biomass of a plant increases, perhaps due to a tissue dilution effect or possibly, a reduced uptake factor.

The tritium activity in leaf tissue in relation to biomass on a chronological basis began with a curvilinear correlation at hour 2 (Figure 28A, \( r = 0.70, p = 0.001 \)), revealing the association between increased tritium activity in the lower biomasses as compared to the level of tritium activity in larger biomass samples. At hour 5, the relationship generally continued but some of the low biomass plants were starting to purge tritium from tissue as evidenced by the low tritium activity in some of the low biomass samples (Figure 28B). The correlation between increased tritium activity and low biomass was very strong in samples collected during hour 7 of the monitoring period (Figure 28C, \( r = 0.94, p < 0.001 \)). No specific association between biomass and tritium activity appeared in samples collected at hour 10 (Figure 28D). In the first sampling on the second day of monitoring (hour 26), the curvilinear correlation between low biomass and higher levels of tritium activity in the leaf tissue was again significant (Figure 28E, \( r = 0.82, p = 0.01 \)). At hour 28, the strength of the correlation again decreased (non-significant) as tritium activity increased in samples ranging between 10-30 g in biomass (Figure 28F, \( r = 0.55, p = 0.18 \)). As on the first
day at hour 10, the mid-afternoon sampling performed at hour 32 on the second day, found no specific relationship between level of tritium activity in leaf tissue and quantity of biomass (Figure 28G). At hour 34 the tritium activity in the leaf tissue and biomass relationship continued to display no specific relationship (Figure 28H). The final sampling (at hour 173) found approximately half of the samples reflecting decreased tritium activity as the biomass increased (Figure 28I).

Tritium Partitioning in the Plants

At the final sampling of the monitoring period, leaf tissue samples were taken and later matched to corresponding root samples obtained during the sectioning of the PVC columns. There was no significant partitioning of tritium activity between roots and shoots. However, in a side-by-side comparison (Figure 29), the more equal partitioning of tritium activity in Larrea revealed possible differences with Atriplex and Tamarix that will need to be confirmed with additional research.

A comparison (ANOVA) of the root/shoot ratios, based on tritium activity in the respective tissue, found Larrea was significantly different from the other species (p = 0.007). Larrea had the smallest root/shoot tritium ratio and Atriplex had the largest ratio (Figure 30).

Comparison of Results from the Hydroponic and Column Studies

Total biomass at harvest for the hydroponic and column studies was significantly different (p < 0.001) as was leaf tritium activity (p < 0.001). However, tritium activity in transpiration was not significant. This suggests the degree to which tritium
partitions into the tissue (possibly organically bound) is related to the amount of biomass accumulated (on a plant). Transpiration tritium activity was plotted against the corresponding total biomass, in each clustering of biomass, transpiration tritium activities ranged from almost zero tritium detected to 40,000 Bq L \(^{-1}\) (Figure 31. Note: multiple samples were taken from each hydroponic tank/column over the entire monitoring period.). Tissue tritium activity in tissue was plotted against the corresponding total biomass and only smaller biomass samples had a large range of tissue tritium activity (Figure 32). The vast majority of samples taken from plants greater than 20 g in total biomass had less than 20,000 Bq kg \(^{-1}\) and plants exceeding 60 g total biomass had less than 5,000 Bq kg \(^{-1}\), possibly displaying a tissue dilution effect and/or greater transpirational loss.

A comparison (ANOVA) made of the dry weight biomass collected from both studies resulted in the finding that tissue type (root or shoot) was significant (\(p = 0.008\)) but growing conditions (hydroponic tank or column) were not significant. In addition, there were significant differences between species (\(p < 0.001\)) and tissue type (\(p < 0.001\)). Based on biomass (Tukey test), *Atriplex* grown in the hydroponic tanks differed significantly from the other four species of the hydroponics study (\(p < 0.001\)), and between *Larrea* grown in the columns (\(p < 0.001\)), *Atriplex* grown in the columns (\(p < 0.001\)) and *Tamarix* grown in the columns (\(p = 0.001\)).

To further highlight commonalities and differences between the studies, results were compared (ANOVA) for *Atriplex* and *Larrea* as these were the species common to the hydroponic and column studies. When transpiration tritium activities from *Atriplex* were compared, the mean hydroponic tritium activity was significantly higher.
than the mean column tritium activity \((p < 0.001)\) by a factor of 42. However, the amount of tritium applied to the column was significantly less (~4 liters per hydroponic tank vs. 100 ml per column). The tritium activity in column tissue was 3.7 times higher than tritium activity in hydroponic tissue \((p = 0.001)\). In contrast, the column biomass was only one-fourth of the biomass attained by the plants grown in the hydroponic study \((p < 0.001)\). For *Larrea*, the biomass grown in the respective studies differed significantly \((p < 0.001)\), at the same time, the level of tritium activity in transpiration and in tissue did not differ.

To clarify if the tritium activity in transpiration over the time course of each study was similar, the tritium activities (collected at each sampling time) for the 5 species of the hydroponic study and the 3 species of the column were compared on an hourly basis with the finding of no significant difference. First day responses in each study were very similar with less than 1,000 Bq L \(^{-1}\) differences between the closest pairs of sampling times. (Sampling times in the two studies were not identical, for comparison, hour 2 of the column study was matched to hour 4 of the hydroponic study.) On the second day, the initial tritium activities in the transpiration were again similar, but differences began appearing with column tritium activities higher (5,000-10,000 Bq L \(^{-1}\)) than hydroponic activities at the two midday samplings. In the late afternoon samplings, the activities in both studies were similar (hydroponic activity, approximately 4,000 Bq L \(^{-1}\); column activity, approximately 3,000 Bq L \(^{-1}\). The transpiration tritium activities were separated by less than 1,000 Bq L \(^{-1}\) for the final samplings (hydroponic study, taken at hour 97; column study, taken at hour 173).
For *Atriplex*, there was no significant difference between transpiration tritium activities of the hydroponic and column studies. The *Atriplex* plants in the column study had a stronger initial response to the single pulse of tritium than plants in the hydroponic study, with the averaged tritium activity in the transpiration of the column study approximately 9,000 Bq L\(^{-1}\) greater than the averaged tritium activity of the hydroponic study (Figure 33). Column tritium activity in transpiration was also greater at the mid-morning sampling by approximately 5,000 Bq L\(^{-1}\), but dropped to nearly undetectable in mid-afternoon while the averaged hydroponic activity dropped to approximately 8,000 Bq L\(^{-1}\). Late afternoon on the first day, the column response was again higher than the hydroponic, by approximately 7,000 Bq L\(^{-1}\). On the second day of monitoring, column tritium activities in the transpiration were higher than hydroponic activities by 2,000 Bq L\(^{-1}\) at the first sampling of the day and increasing to approximately 4,000 Bq L\(^{-1}\) higher by mid-afternoon. At the final sampling, while the hydroponic study had approximately 2,000 Bq L\(^{-1}\) in the transpiration and the column study had nearly zero tritium activity, it should be noted that the volume of tritiated fluid available for transpiration was approximately 4 liters in the hydroponic study versus 100 ml of tritiated solution in the column study. Potentially, in the column study, all available tritiated fluid had been transpired by the final sampling, thus accounting for the near zero tritium activity.

In *Larrea*, there was a marginally significant difference in the transpiration tritium activities (p = 0.051). At the initial sampling following application of the tritiated solution, the hydroponic *Larrea* plants had a response greater than the column plants by approximately 14,000 Bq L\(^{-1}\) and again at the mid-morning sampling (Figure 34).
Less than 2,000 Bq L\(^{-1}\) separated the *Larrea* studies during the two afternoon samplings, with the column transpiration tritium activities less than the tritium activities measured in the hydroponic study. The first sampling of the second day found column activity to be slightly higher, but only by approximately 1,000 Bq L\(^{-1}\). At the mid-morning sampling, transpiration tritium activity in the column-grown *Larrea* was approximately 33,000 Bq L\(^{-1}\) compared to a tritium activity of 10,000 Bq L\(^{-1}\) for the hydroponic-grown *Larrea*.

A marginally significant difference was found (p = 0.067) for hourly tissue tritium activities for the 5 species of the hydroponic study and the 3 species of the column study (ANOVA). The column study activity was higher by a minimum of 5,000 Bq kg\(^{-1}\) during the first three samplings on day 1 of the monitoring. By late afternoon, the tritium activity in hydroponic tissue exceeded the column activity, although by the next morning the tritium activity in column tissue was again the highest. On the second day of monitoring, tritium activities in column tissue were higher for each of the comparable sampling times; going from a small difference of approximately 3,000 Bq kg\(^{-1}\) at the early morning sampling and increasing over the day to a difference of approximately 12,000 Bq kg\(^{-1}\) at the late afternoon sampling. The final samplings at hour 97 of the hydroponic study and hour 173 of the column study were separated by approximately 5,000 Bq kg\(^{-1}\), with the tritium activity highest in column tissue. This difference may be related to column plant tissue being exposed to the tritiated solution for almost 100 hours longer than hydroponic tissue, potentially allowing more time for organic binding of \(^3\)H in less easily-exchanged positions of organic molecules in plants grown in columns. Application of tritiated solution to the columns exposed...
only growing roots in specific zones to tritium, whereas in the hydroponic tanks, the entire root system was equally exposed to tritiated solution.

While the Larrea data from the hydroponic study was not robust enough on the first day of monitoring to permit a good comparison, the second day of monitoring (from each study) could be compared. The final average column tissue tritium activity was approximately 10,000 Bq kg$^{-1}$ compared to the final averaged hydroponic activity of 5,000 Bq kg$^{-1}$ (Figure 35).

For Atriplex, there was a marginally significant difference between the studies ($p = 0.0878$). The initial sampling of Atriplex (on day 1 of monitoring) found higher tissue tritium activity in the column study. At mid-morning of the first day of monitoring, the hydroponic plants had higher tissue tritium activity, and by mid-afternoon the column study plants again had the higher tissue tritium activity; although the difference was never more than approximately 2,000 Bq kg$^{-1}$ over the entire day (Figure 36). In the hydroponic study, the average tissue tritium activity was approximately 4,000 Bq kg$^{-1}$ higher than the column tissue tritium activity. On the second day, column tissue tritium activity was higher than hydroponic tissue tritium activity at each sampling time, with the early morning, mid-morning and early afternoon sampling differing by approximately 2,500 Bq kg$^{-1}$. By late afternoon, the average tissue tritium activity of the column study was 8,000 Bq kg$^{-1}$ compared to the average hydroponic tissue tritium activity of 2,000 Bq kg$^{-1}$. The final average tissue tritium activity from the column study (collected at hour 173 of the monitoring period) was approximately 10,000 Bq kg$^{-1}$. This activity was twice the tissue tritium activity
(5,000 Bq kg\(^{-1}\)) found in the final sampling of the hydroponic study, collected at hour 97 of the monitoring period.
CHAPTER 5

DISCUSSION

The primary goal of this study was to identify the potential of native and exotic plant species to function as sentinels of tritium contamination originating from low-level radioactive waste sites. In addition to identifying tritium uptake potential, this study also provided an opportunity to observe and compare the relative growth rate of each species, during daily maintenance of plants and growing systems. Evaluation of a species' ability to provide shoot biomass for transpirational capture and tissue samples was derived from the tritium activity data and the growth observations. The hydroponic setting provided direct contact between the root systems and tritiated water, allowing ease of uptake while the root systems of plants in the column study were able to develop a less confined root system and in the case of Tamarix, a suberized root system that provided a different uptake environment. Atriplex vigorously responded to having the root system immersed in a nutrient solution, developing a large root system and luxuriant canopy. From the time of planting in the columns until harvest six months later, Atriplex developed a shoot biomass that was slightly less than Tamarix, illustrating Atriplex's rapid growth habit as the Tamarix were almost two years old at time of harvest. The column-grown Atriplex only achieved 25% of the biomass produced by the hydroponic grown Atriplex; however, the column growth was probably more reflective of the biomass which would be
expected under field conditions. In a monitoring design requiring establishment of
monitoring plants from seed, based on growth observed in this study, *Atriplex* has the
potential to provide plants large enough for sampling within one year. In both studies,
*Larrea* produced less root biomass and less shoot biomass than *Atriplex*, reflecting the
slower growth habit of this long-lived perennial. On a daily basis, the hydroponic
*Atriplex*, and to a lesser extent the column plants, shed leaves suggesting that under
field conditions when a daily record of a rapidly moving contamination plume needs
to be documented; sacrificing small portions of the existing canopy would not cause
irreparable damage to the plant. The evergreen leaves of *Larrea* are retained longer
and would provide a better history of contamination when monitoring is less frequent.
*Ephedra* also exhibited a slow growth habit and would be capable of providing long-
term, low maintenance monitoring, but the difficulty of dissolving the shoot tissue in
preparation for liquid scintillation counting would make it a poor choice.

Evaluating the species solely on the basis of root production, *Pleuraphis* extended
numerous long roots the bottom of the hydroponic tank. Mining soil water in the
profile would be dependant on development of a deep root system and in the drier soil
environment, the process is likely to be slower than in the hydroponic tanks.
However, pre-transfer root development and the rapid root growth demonstrated in
nutrient solution, suggest *Pleuraphis* would potentially be a good monitor for tritium
contamination (dependant on root depth attained under field conditions). *Atriplex*
roots so quickly filled their hydroponic tanks that competitive effects between the
three plants per tank were being observed prior to application of the tritium treatments,
while in the column study *Atriplex* roots filled the upper 20 cm of soil and also
extended the 3 m length of the column. These observations suggest that *Atriplex* is capable of extracting moisture within the upper 3 meters of a soil profile. However, it is recommended that incorporation of *Atriplex* into a monitoring scheme on an earthen cap be done only if an effective capillary barrier is part of the engineering design, to hinder *Atriplex* roots from growing into the trench (Nyhan *et al.*, 1990), and thus preventing the possible translocation of tritium into the upper soil level and into the atmosphere via water uptake and transpirational loss (Kennedy *et al.*, 1982).

Transpiration, in the presence of a strong sink for uptake, is the primary mechanism governing the extent to which a plant is capable of removing tritium from a nutrient solution or soil. Based on the rate of transpiration, this will also affect the extent to which there is time for the tritium to become incorporated into plant tissue; hence, a strong transpiration stream decreases opportunity for organic binding (Belot 1986, Amano and Garten 1991, Murphy 1993). In the large, hydroponic *Atriplex* plants, a strong relationship between increasing transpiration rate and decreasing tritium activity in the leaf tissue was observed. This relationship was less robust on the second day of monitoring as evidenced by increasing tritium activity in the leaf tissue. However at the end of monitoring, *Atriplex* had the lowest tritium activity in its leaf tissue of the five species tested in the hydroponic study. Even though differences existed in the uptake dynamics of tritium located in soil as compared to tritium in a nutrient solution, the link between a higher transpiration rate (inferred based on larger leaf surface area) and a lower tritium activity in the leaf tissue of column *Atriplex* was maintained for two days following input of a tritiated solution. The large difference between tritium activity in the transpiration samples and tritium activity in tissue
samples collected from hydroponic *Atriplex* clearly revealed the uptake and clearing of tritium activity from tissue by these plants, demonstrating that this species has excellent potential to function as a sentinel of contamination when transpiration samples are the preferred method of monitoring. *Atriplex* had no significant difference in response to level of tritium treatment (in both studies). This insensitivity is another plus for use of this species in a sentinel capacity, as the two studies have demonstrated that any contamination of 100 tritium units or higher within reach of the *Atriplex* root system can be effectively monitored through leaf/shoot sampling. This sampling technique has the advantage of allowing a technician to remain out-of-direct contact with an inground contamination flow.

A higher sensitivity of *Larrea* than the other species to the 100 TU level of tritium treatment was observed in the hydroponic study and also in the column study, suggesting that it may be a species trait and not just a function of the very small biomass that occurred in the hydroponic study. A cyclic accumulation and release of tritium activity in leaf tissue, independent of whole plant transpiration rate, also distinguished *Larrea* from the other species. The early morning decrease of tritium activity in leaf tissue, followed by a 24-hour accumulation, was observed in both studies. This suggests the transpiration pattern of this species sets up a situation in which uptake of tritiated water is more rapid than evacuation of tritiated water from leaf tissue via transpirational flow. The lack of a tight correlation between increasing transpirational tritium activity as the whole plant transpiration rate increased in the hydroponic study may substantiate this conclusion, but the low biomass of the hydroponic plants and the experimental conditions must be factored into the
evaluation. *Larrea* plants experienced a more difficult growth environment in the hydroponic tanks than in the columns, although the shoot portion of hydroponic and column plants were exposed to similar ambient conditions. Leaf longevity of *Larrea* plants was shorter in the hydroponic tanks than in the columns, this may have been a result of thermal conditions in the root environment. Temperature of the nutrient solution frequently exceeded 32° C in response to the ambient air temperatures each afternoon. The soil in the columns may have provided greater insulating capacity against the afternoon heat, resulting in less thermal stress to root systems of *Larrea* plants grown in columns.

In the column study, which was more similar to field conditions than the hydroponic study, tritium activity in *Larrea* transpiration samples increased at the times when transpiration rates were predicted to reach daily maximum and a positive relationship between low biomass and higher tritium activity in transpiration samples (compared to the other species) was detected. This suggests the slow accumulation of biomass in *Larrea* does not preclude it from being an effective sentinel of tritium contamination. Further corroboration of the tritium retained in leaf tissue came from the small root-to-shoot tritium activity ratio of hydroponically-grown *Larrea*. This finding substantiates the conclusion that for monitoring purposes, *Larrea* would be a good candidate for leaf tissue sampling or root tissue sampling.

Tritium root and shoot ratios in *Atriplex* and *Tamarix* were the result of lower tritium activity in root tissue than tritium activity in leaf/shoot tissue. The movement of tritium from nutrient solution to atmosphere can be conceived as occurring in three phases: first, uptake by and movement through root tissue; second, movement into and
through the shoot vascular system; third, exit from stomata to atmosphere via transpiration. The hydroponic root samples were collected at approximately 100 hours post-tritium introduction, providing sufficient time for tritium to be cleared from tissue (by transpiration) according to the model developed by Brudenell et al (1997). A more likely explanation is that tritium activity in the root tissue was approaching equilibrium with tritium activity in the nutrient solution; activity in the nutrient solution samples (collected same time as root samples) was below the level of detection, having been depleted during the monitoring period. The accumulation of tritium activity in the leaf/shoot tissue resulted because the transpiration rate was of insufficient strength to keep the tritiated solution moving through the vascular system. Reliance on data from only root samples, if unknowingly taken after passage of a contamination plume, would indicate no contamination and possibly, an interpretation of no tritium movement from the burial site. Reliance on data from only leaf tissue could lead to overestimation of the problem due to high tritium activity through accumulation. In the event of finding high tritium activity when sampling shoot tissue, it is recommended root samples also be collected to obtain a more complete picture of the level of contamination.

The response of *Bromus* to the tritiated solution was slower than in *Atriplex*, as the lowest leaf tissue tritium activity in the first two days of monitoring coincided with the overnight decrease in whole plant transpiration, following high tritium activities in leaf tissue for most of the first day. Thereafter, indications were for an increasing level of tritium activity in leaf tissue in parallel with an increasing transpiration rate at the whole plant level, though this latter rate did not have significant variation over the
course of monitoring. The gradual increase in tritium activity of transpiration samples during the second day, reaching the highest activities in the afternoon was evidence of a 12-24 hour delay in response to a tritium pulse after which tritium activity was higher in transpiration than in leaf tissue. The inference that in *Bromus* there was a statistically significant separation of tritium in transpiration based on TU treatment should be accepted with caution. As a sentinel plant, *Bromus* has the potential to be useful under conditions of cooler temperatures and higher soil moisture contents. (Conditions usually found during early-to-mid spring in the hot deserts. *Bromus* is a short-lived annual, generally 6-10 weeks from germination to senescence). The root system could readily sample the upper several centimeters of soil (a limitation if contaminant flow is occurring only at deeper depths), and though the proportion of tritium activity in leaf tissue relative to root tissue is more variable than in *Larrea*, the ease of removing this plant to examine root tissue is a valuable trait.

*Ephedra* exhibited little variation in response to the input of a tritiated solution; there was no significant difference between gradations within any of the measured parameters (i.e., the amount of tritium activity in tissue, sampling times, response to the two tritium treatments, the amount of whole plant transpiration across a day, or tritium activity in transpiration samples). When the root to shoot tritium activities were compared for the hydroponic species, *Ephedra* had higher tritium activities in the roots. *Ephedra* responded more quickly to input of the tritiated solution than the other species, as evidenced by higher tritium activity in leaf tissue at the first sampling (on the first day of the monitoring), as well as in the samples taken after lower overnight transpiration rates. The second sampling time was associated with a decrease in stem
tritium activity (below the initially detected tritium activity) while plant transpiration was increasing, possibly preventing a build-up of tritium activity in the stem tissue. (Note: Ephedra has photosynthetic stems and is leafless.) However, following this second sampling time, we hypothesize that the transpiration stream imported more tritiated water into the Ephedra tissue than could exit in the vapor phase via the stomata, leading to a subsequent rise in tritium activity in stem tissue and roots. The results suggest that Ephedra would be one of the first species to provide an alert to tritium contamination and appears to be more likely to retain traces of tritium activity longer than Atriplex, making Ephedra a good choice for those situations where monitoring would be less frequent. However, the tendency to maintain high levels of tritium activity in root tissues might be of concern, as this could be a source of further contamination if there is possible flow and exchange away from the plant.

In Pleuraphis, the lack of a robust data set made it difficult to establish relationships between tritium activity in leaf tissue, tritium activity in transpiration samples and whole plant transpiration. Statistical analysis indicated that Pleuraphis demonstrated less sensitivity to the 100 TU treatment than Bromus, Atriplex and Ephedra. There were no outstanding reasons derived from this study to recommend this plant as a viable sentinel of tritium contamination.

Interpretation of the experimental results for Tamarix requires an understanding that these plants entered the study with well-developed, suberized root systems and well-developed, lignified shoots that influenced uptake of the tritiated solution and subsequent purging of tritium from the plant tissue. Results from analysis of the root tissue indicated that if tritium contaminated soil water encountered the root system of
Tamarix in the unsaturated zone; tritium will be slowly translocated upwards into root tissue above the source of contamination into aboveground biomass. Slow tritium uptake imposed by the suberized layer of root tissue led to a slow response in leaf tissue tritium activities on the first day of monitoring, as evidenced by a variation of less than 2,000 Bq kg⁻¹. However, on the second day of monitoring, the peak activity slightly exceeded 10,000 Bq kg⁻¹, which yielded a variation of approximately 6,000 Bq kg⁻¹ between the highest and lowest tritium activities of the day. On the fifth day of monitoring, the tritium activity of leaf tissue was only 3,000 Bq/kg less than the peak value detected on day 2. The response observed suggests analytical and statistical models could be developed to explain variation in leaf tissue tritium activity relative to a tritium contamination event. Though statistical analysis did confirm that tritium activity in transpiration was not significantly different from the tritium activity in leaf tissue for Tamarix, average tissue activity did exceed average transpiration activity. Since tritium activity in tissue is a function of whole plant transpiration (Amano et al., 1995) and this study found an association between decreasing tritium activity in leaf tissue as the biomass of a plant increases, evaluating the latter in light of the uptake restriction imposed by the root suberin layer suggests the relationship between transpiration, tissue tritium activity and biomass is moderated by root adaptations to the local environment. The two studies comprising this project identified that plant response to a single pulse of tritiated solution is a complex interaction of root development in desert plants (Wallace et al., 1974, Stringer et al., 1989), species-specific uptake dynamics (Zeng et al., 1996), and the manner in which transpiration is moderated by the mass of aboveground biomass.
Suggestions for Further Research

Further study could clarify how root development, species-specific uptake dynamics, and moderation of transpiration by aboveground biomass interact in species adapted to arid ecosystems. The encountering of tritium under field conditions is likely to be a situation of extended availability, and the plant response may not be identical to the response observed under single-pulse conditions. An experimental design in which a longer, constant tritium source is provided to the plants would identify differences between single-pulse dynamics (Young et al., 1970, Kline et al., 1976, Belot 1983) and constant source dynamics of transpirational uptake (Dinner et al., 1980, Baumgärtner et al., 2001) and isotope exchange within various tissue types (Raney and Vaadia 1965, Garland and Ameen 1979, Belot 1986, Takashima et al., 1987). Under field conditions, growing plants long enough to obtain specimens with more mature root and shoot systems than generally used in this project would help clarify some of the questions about the relationship between biomass and tritium activity in tissue. Does sentinel ability change with plant age and do arid zone adaptations such as suberization eventually make a desert plant a poor sentinel? This question is especially pertinent to woody species such as Larrea and Atriplex. The sentinel plants used in the hydroponic/column studies were young and growing; at Maxey Flats, Kentucky, mature trees native to a mesic environment were used as sentinels (Rickard & Kirby 1987). The Tamarix data suggest root suberization in those 2-year old plants influenced uptake dynamics of the tritiated solution. Whether plant age is a pertinent factor for sentinel ability in arid zone plants could be explored.
in an experiment using plants ranging in age from young seedling to fully-developed mature plants.

Taking into consideration the aggressive growth of *Atriplex* roots (Stringer et al., 1989), a recommended follow-up study would consist of growing each of the species (grown in this project) above simulated capillary barriers to ascertain if the barriers would effectively prevent root intrusion into stored wastes (Nyhan et al., 1990), thereby preventing translocation of safely-stored radioactive material (Kennedy et al., 1982). As translocation of a tritiated source is intimately linked to the quantity of transpirational flow (Schulz 1991, McIntyre 1994), which is in turn governed by environmental and growth/photosynthetic demands on water (Woods and O’Neal 1964), measurement of the transpirational flow through the use of sap flow gauges in any of the studies suggested above has the potential to elucidate the relationship between diurnal cycles of tritium and water uptake (Devitt et al., 1993, Sala et al., 1996).

Based on the difficulties that were encountered in this study associated with attaining complete decolorization of leaf tissue samples containing large amounts of chlorophyll, resulting in chemiluminescence when the samples were counted with liquid scintillation, a study to improve the bleaching techniques and development of color quenching standards for species such as *Tamarix* and *Ephedra* would be suggested (Lee, 1979, Burrell and Brunt 1981, Takiue et al., 1984, 1991, Pujol 1999).

The final suggested study would be a detailed examination of the isotopic discrimination, exchange of carboxyl hydrogens and subsequent organic binding of tritium through exchange of hydroxyl hydrogens, as driven by the evapotranspiration
processes of desert plants, to partition the effects governing tritium activity in leaf
tissue into those mechanisms associated with quantity of biomass and the mechanisms
associated with the differential discrimination of C$_3$, C$_4$ and CAM physiology (Smith
and Ziegler 1990, Diabaté and Strack 1993, Kim and Baumgärtner 1993, Baumgärtner
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Table 1. The average total biomass produced by the five species grown in the hydroponic study and the three species grown in the column study.

<table>
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<th>Species</th>
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<td>hydroponic</td>
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<td>0.229</td>
<td>0.2</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>hydroponic</td>
<td>0.3</td>
<td>0.340</td>
<td>0.2</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Atriplex shoot column 17.6 5.686 17.6 1.895
Larrea shoot column 2.1 1.481 2.1 0.494
Tamarix shoot column 25.3 8.682 25.3 2.894

1 The Least Significant Difference (LSD<sub>0.05</sub>) for the hydroponic study was calculated as 19.80.
2 The Least Significant Difference (LSD<sub>0.05</sub>) for the column study was calculated as 32.25.
Table 2. Tritium Activity of Leaf Tissue in the Hydroponic Study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Significance of Sampling Time</th>
<th>Sampling time</th>
<th>p-value</th>
<th>Significance of Tritium Treatment</th>
<th>Tritium treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atriplex</td>
<td>significant</td>
<td></td>
<td>0.045</td>
<td>N.S.</td>
<td></td>
<td>0.542</td>
</tr>
<tr>
<td>Bromus</td>
<td>N.S.</td>
<td></td>
<td>0.215</td>
<td>N.S.</td>
<td></td>
<td>0.168</td>
</tr>
<tr>
<td>Ephedra</td>
<td>N.S.</td>
<td></td>
<td>0.881</td>
<td>N.S.</td>
<td></td>
<td>0.742</td>
</tr>
<tr>
<td>Larrea</td>
<td>significant</td>
<td></td>
<td>0.032</td>
<td>N.S.</td>
<td></td>
<td>0.083</td>
</tr>
</tbody>
</table>

Note: N.S. is used to indicate 'not significant'.
Table 3. Whole Plant Transpiration in the Hydroponic Study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Significance of Sampling Time</th>
<th>p-value</th>
<th>Significance of Tritium Treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atriplex</td>
<td>significant</td>
<td>0.010</td>
<td>N.S.</td>
<td>0.164</td>
</tr>
<tr>
<td>Bromus</td>
<td>N.S.</td>
<td>0.098</td>
<td>N.S.</td>
<td>0.058</td>
</tr>
<tr>
<td>Ephedra</td>
<td>N.S.</td>
<td>0.444</td>
<td>N.S.</td>
<td>0.178</td>
</tr>
<tr>
<td>Larrea</td>
<td>significant</td>
<td>0.181</td>
<td>N.S.</td>
<td>0.140</td>
</tr>
</tbody>
</table>

Note: N.S. indicates 'not significant'.
Table 4. Tritium Activity in Transpiration Samples Collected in the Hydroponic Study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling time</th>
<th>Significance of Sampling Time</th>
<th>p-value</th>
<th>Significance of Tritium Treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atriplex</td>
<td>N.S.</td>
<td>0.246</td>
<td>N.S.</td>
<td>0.928</td>
<td></td>
</tr>
<tr>
<td>Bromus</td>
<td>significant</td>
<td>0.014</td>
<td>N.S.</td>
<td>0.778</td>
<td></td>
</tr>
</tbody>
</table>

Hours differing from each other

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th></th>
<th>p-value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>9 and 37.5</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5 and 37.5</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 and 37.5</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 and 37.5</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 and 37.5</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.5 and 37.5</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.5 and 37.5</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97 and 37.5</td>
<td>0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ephedra</td>
<td>N.S.</td>
<td>0.174</td>
<td>N.S.</td>
<td>0.559</td>
<td></td>
</tr>
<tr>
<td>Larrea</td>
<td>N.S.</td>
<td>0.130</td>
<td>N.S.</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td>Pleuraphis</td>
<td>N.S.</td>
<td>0.656</td>
<td>N.S.</td>
<td>0.824</td>
<td></td>
</tr>
</tbody>
</table>

Note: N.S. indicates 'not significant'.
Figure 1. A. A comparison of the average shoot biomass produced by the five desert species grown in the hydroponic study. B. *Atriplex* is removed from this comparison to more clearly illustrate the relative biomass of the other four species.
Figure 2. A. A comparison of the average root biomass produced by the five desert species grown in the hydroponic study. B. *Atriplex* is removed from this comparison to more clearly illustrate the relative biomass of the other four species.
Figure 3. Evapotranspiration relative to dry weight biomass during the 97-hour monitoring period of the hydroponic study.

\[ Y = 291.9 + 22.9X, \quad r^2 = 82^{***}, \quad n = 14\]
Figure 4. For all species grown in the hydroponic study, the tritium activity in leaf/stem tissue was averaged for each sampling time (of the 97-hours monitoring period). The transpiration rates of sampled tanks were also averaged for each sampling time.
Figure 5. The average tritium level in leaf tissue compared to the rate of transpiration during the 97-hour monitoring of the hydroponic study.
Figure 6 (continued). The average tritium activity in transpiration samples compared to the rate of whole plant transpiration during the 97-hour monitoring of the hydroponic study.
Figure 7. An hourly comparison of tritium activity in tissue and transpiration samples for all the species grown in the hydroponic study. Leaf tissue samples and transpiration samples are from the same hydroponic tank and were collected at the same sampling time.
Figure 8. An hourly comparison of tritium activity in corresponding leaf tissue and transpiration samples, collected from the same hydroponic tank and at the same sampling time. A. Samples from Day 1. B. Samples from Day 2. C. *Atriplex* is plotted separately, and represents the majority of low activity samples from the first two days of monitoring.
Figure 9. The tritium activity detected in transpiration samples (Bq/L) in relation to the total biomass harvested during monitoring of the hydroponic study.
Figure 10A. The highest tritium activity detected in transpiration samples collected in the hydroponic study as a function of the total biomass at harvest.

Figure 10B. The tritium activity in transpiration samples from the hydroponic study relative to biomass, and distinguished by species. *Atripllex* was deleted from this depiction to better illustrate the range of tritium activity in the species that averaged less than 10 g in biomass.
Figure 11. The tritium activity in transpiration samples (for all species) on Day 1 of monitoring, in relation to the total biomass that was harvested in the hydroponic study.
Figure 12. The tritium activity in transpiration samples (all species) on Day 2 of monitoring, relative to the total harvested biomass in the hydroponic study.
Figure 12. (continued). The tritium activity in transpiration samples (all species) on Day 2 of monitoring, relative to the total harvested biomass in the hydroponic study.
Figure 13. The highest tritium activity that was measured in tissue relative to the total harvested biomass. All species grown in the hydroponic study and both tritium treatments are included.
Figure 14. The tritium activity in tissue samples in relation to the total biomass, collected during monitoring of the hydroponic study.
Figure 15. Tritium activity in leaf and stem tissue (all species) on the first day of monitoring relative to the total biomass harvested in the hydroponic study.
Figure 16. Tritium activity in leaf and stem tissue (all species) on the second day of monitoring, in relation to the total biomass harvested in the hydroponic study.
Figure 16 continued. Tritium activity in leaf and stem tissue ((all species) on Day 2 of monitoring, in relation to total biomass harvested in the hydroponic study.
Figure 17. The ratio of tritium activity in root tissue to the tritium activity in leaf and stem tissue (for each species) during the 97-hour monitoring in the hydroponic study.
Figure 18. Soil samples were collected from each column immediately prior to application of tritium treatments. An average gravimetric water content was calculated for each species in the column study.
Figure 19. The average tritium activity in *Atriplex* root tissue at the four sampling positions following the final harvest in the column study. Sampling was performed at 17 cm above the input port, at the input port, 17 cm below the input port and at the end of each column.
Figure 20. The average tritium activity in *Larrea* root tissue at the four sampling positions following the final harvest in the column study. Sampling was performed at 17 cm above the input port, at the input port, 17 cm below the input port and at the end of each column.
Figure 21. The average tritium activity in *Tamarix* root tissue at the four sampling positions following the final harvest in the column study. Sampling was performed at 17 cm above the input port, at the input port, 17 cm below the input port and at the end of each column.
Figure 22. The average tritium activities in the leaf tissue at each sampling time. Atriplex, Larrea and Tamarix were grown in soil columns and tritium applied in a single pulse of tritiated water at one meter below the soil surface.
Figure 23. The average tritium activity in transpiration at each sampling time during monitoring of the column study. *Atriplex*, *Larrea* and *Tamarix* were grown in soil columns and tritium applied in a single pulse of tritiated water at one meter below the soil surface.
Figure 24. The tritium activity in transpiration was compared to the tritium activity in leaf tissue during the 173-hour period following a single pulse of tritiated water applied one meter below soil surface in the column study. A. This is a comparison of all three species grown in column study. B - D. The three species are plotted individually.
Figure 25A. The highest tritium activity in transpiration samples collected during monitoring of the column study. *Larrea* was distinguished from *Atriplex* and *Tamarix* by higher tritium activity per unit of biomass. *Atriplex* and *Tamarix* had similar tritium activity and similar biomass.

Figure 25B. The tritium activity in transpiration samples (by species) in relation to biomass collected during monitoring of the column study. *Larrea* differed from *Atriplex* and *Tamarix* because of high tritium activities and low biomass. Some of the *Tamarix* differed because of high biomass and low tritium activities.
Figure 26 A-D. Tritium activities in transpiration samples (for all species) in relation to total biomass harvested in the column study.
Figure 26 E-G. Tritium activity in column transpiration samples (for all species) in relation to total biomass harvested in the column study. The correlation in Hour 28 was significant (p = 0.05), Hour 173 was non-significant (p = 0.05).
Figure 27A. The highest tritium activity found in tissue samples (by species) in relation to biomass collected during monitoring of the column study.

Figure 27B. The tritium activity in tissue samples (by species) in relation to biomass collected during monitoring of the column study.

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FIGURE 28. At each sampling time, the tritium activity found in tissue samples (for all species) in relation to total biomass harvested in the column study.
FIGURE 28 (continued). Tissue tritium activity (all species) in relation to total biomass harvested.
Figure 29. The distribution of tritium activity in root tissue and in shoot tissue of species grown in the column study.
Figure 30. The averaged ratio between tritium activity in root tissue and tritium activity in shoot tissue for each species grown in the column study.
Figure 31. A comparison between tritium activity in transpiration samples from the hydroponic study and tritium activity in transpiration samples from the column study.
Figure 32. A comparison of the hydroponic and column studies for tritium activity in tissue samples (Bq/kg) relative to total harvested biomass (g).
Figure 33. A comparison of the hydroponic and column studies for tritium activity in Atriplex transpiration samples (Bq/L) relative to total harvested biomass (g).
Figure 34. A comparison of the hydroponic and column studies for tritium activity in *Larrea* transpiration samples (Bq/L) relative to total harvest biomass (g).
Figure 35. A comparison of the hydroponic and column studies for tritium activity in *Larrea* tissue samples (Bq/kg) relative to total harvested biomass (g).
Figure 36 A comparison of the hydroponic and column studies for tritium activity in *Atriplex* tissue samples (Bq/kg) relative to total harvested biomass (g).
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Publications:

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