Spotted knapweed (Centaurea maculosa Lam) : water, nutrients, plant competition, bacteria, and the seed head fly (Urophora affinis Frnfd.)
by Stephen Anthony Kearing

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Spotted knapweed, Centaurea maculosa Lam., is considered fay many to be the number one noxious weed in western Montana. A hydroponics study was conducted in Bozeman, MT to quantify water and nutrient uptake of spotted knapweed and two grass competitors. Spotted knapweed, western wheatgrass, Pascopymm smithii (Rydb.) Love, and a crested wheatgrass hybrid, Agropyron cristatum (L.) Gaert X Agropyron desertorum (Fisch. ex Link) Schult., were grown in a complete randomized block design, each in individual hydroponic drip systems with an inert rock wool media. The pots were weighed at each interval to estimate water use.

Mean concentrations remaining in the system for nitrogen, phosphorous, and potassium were regressed against mean cumulative water use for each treatment.

Spotted knapweed and western wheatgrass had similar slopes for nitrogen and potassium concentrations that were significantly lower than crested wheatgrass (t-test, $P <= 0.05$), suggesting that knapweed and western wheatgrass absorb nitrogen and potassium more efficiently than crested wheatgrass. Conversely, crested wheatgrass had a significantly lower slope for concentrations of phosphorous remaining in the solution (t-test, $P = 0.01$), suggesting crested wheatgrass absorbs more phosphorous per ml water than western wheatgrass or spotted knapweed. Spotted knapweed used more water throughout the experiment ($P = 0.01$), with differences being greatest during bolting. The combination of water and nutrient uptake rates help to explain spotted knapweed's ability to compete for resources.

The effects of fertilization and plant competition on spotted knapweed, dry weight and gall density of Urophora affinis Frnfd. (Diptera: Tephritidae) were investigated in a field study in Bozeman, MT. Spotted knapweed was grown in a complete randomized block design with two grass competitors: Rosana western wheatgrass and Hycrest crested wheatgrass. Treatments also included the application of three rates of phosphorus fertilizer (60, 120, 240 mg/L). Bouquets of dried spotted knapweed flower heads, containing Urophora affinis, were placed within the test plot to allow for natural eclosion and attack of the developing spotted knapweed flower buds.

Spotted knapweed plants growing with a grass treatment were significantly smaller ($P = 0.00$), while phosphorous fertilization resulted in significantly higher spotted knapweed dry weight ($P = 0.01$). However, significantly more U. affinis galls per seed head were found in the control without phosphorous, suggesting smaller, less vigorous plants may be more susceptible to attack by U. affinis. These results also suggest the importance of evaluating fertilization impact on both the target weed and biocontrol agents before such activities are widely adopted.

Using Koch's postulates, a bacterial pathogen Pseudomonas syringae pv syringae van Hall was isolated from diseased spotted knapweed stem and bud tissue in the field project. Usually considered an epiphyte, it is suggested that the bacterium took advantage of environmental conditions, a cold and wet

season, and high levels of bud wounding from the artificially inflated U. affinis population.

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Master of Science

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Entomology

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 1996

APPROVAL

of a thesis submitted by

Stephen Anthony Kearing

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 9 May 1996

ACKNOWLEDGEMENTS

I offer sincere thanks to Dr. Robert M. Nowierski, who advised me during the past three years. His guidance directed my approach to problem solving and provided inspiration.

A hearty thank you goes to my committee members, Drs. Kevin O'Neill and Earl Skogley. Their comments and encouragement both in and out of the classroom were invaluable.

Bryan Fitzgerald showed extreme patience and provided daily training throughout my development as a graduate student. Dr. Zheng Zeng provided computer expertise that brought me back from the great abyss. Drs. William Grey and Donald Mathre were extremely generous with their time, equipment, and laboratory space.

I also need to thank the following people: Ann Kennedy, Chuck Quimby, Norman Rees, Douglas Ambach, Jake Stoddart, Pete Fay, Bruce Maxwell, Roger Sheley, David Baumbauer, Robert T. Grubb, and the individuals at M.S.U.'s Soil Testing Laboratory.

A special thanks to my family, Susan Bousum, and Holmes. I would not have finished without them.

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GLOSSARY OF TERMS AND ABBREVIATIONS

biological control (biocontrol): the use of an insect, **pathogen**, or other living organism to control a pest.

causal complex: organisms and environmental factors responsible for disease.

crested wheatgrass: in this paper refers to a Hycrest crested wheatgrass hybrid, *Agropyron cristatum* (L.) Gaert X *Agropyron desertorum* (Fisch. ex Link) Schult.

competition: mutually adverse effects of organisms which use a resource in short supply.

epiphyte: a plant growing upon another plant, usually refers to cases where no **parasitic** or **symbiotic** relationship is involved.

evapotranspiration: water used in transpiration and evaporation.

GC-FAME: Gas Chromatography Fatty Acid Methyl Ester. A bacteria identification technique based on fatty acid composition.

GDD: growing degree-days.

infection: establishment of the **pathogen** within the host following **penetration**.

incitant: organism that incites a disease under the influence of other factors.

parasite: an organism that subsists whole or in part upon living tissue.

pathogen: an agency that incites disease.

pathogenesis: the process or chain of events by which disease development takes place.

pathogenicity: the property of a microorganism by which it may become a part of the **causal complex.**

penetration: initial invasion of the host by an organism.

pv (pathovar): pathogenic variety.

sporadic disease: occur at irregular intervals and locations and in relatively few instances.

susceptibility: condition of plant in which it is normally subject to attack by a given pathogen.

symbiont: two organisms association that is mutually beneficial.

western wheatgrass: in this paper refers to a Rosana western wheatgrass variety, *Pascopyrum smithii* (Rydb.) A Love.

Water Use Efficiency (WUE): ratio of dry matter produced to water used in evapotranspiration.

ABSTRACT

Spotted knapweed, *Centaurea maculosa* Lam., is considered by many to be the number one noxious weed in western Montana. A hydroponics study was conducted in Bozeman, MT to quantify water and nutrient uptake of spotted knapweed and two grass competitors. Spotted knapweed, western wheatgrass, *Pascopyrum smithii* (Rydb.) Love, and a crested wheatgrass hybrid, *Agropyron cristatum* (L.) Gaert X *Agropyron desertorum* (Fisch. ex Link) Schult., were grown in a complete randomized block design, each in individual hydroponic drip systems with an inert rock wool media. The pots were weighed at each interval to estimate water use. Mean concentrations remaining in the system for nitrogen, phosphorous, and potassium were regressed against mean cumulative water use for each treatment.

Spotted knapweed and western wheatgrass had similar slopes for nitrogen and potassium concentrations that were significantly lower than crested wheatgrass (t-test, $P <= 0.05$), suggesting that knapweed and western wheatgrass absorb nitrogen and potassium more efficiently than crested wheatgrass. Conversely, crested wheatgrass had a significantly lower slope for concentrations of phosphorous remaining in the solution (t-test, $P = 0.01$), suggesting crested wheatgrass absorbs more phosphorous per ml water than western wheatgrass or spotted knapweed. Spotted knapweed used more water throughout the experiment ($P = 0.01$), with differences being greatest during bolting. The combination of water and nutrient uptake rates help to explain spotted knapweed's ability to compete for resources.

The effects of fertilization and plant competition on spotted knapweed, dry weight and gall density of *Urophora affinis* Frnfd. (Diptera: Tephritidae) were investigated in a field study in Bozeman, MT. Spotted knapweed was grown in a complete randomized block design with two grass competitors: Rosana western wheatgrass and Hycrest crested wheatgrass. Treatments also included the application of three rates of phosphorus fertilizer (60, 120, 240 mg/L). Bouquets of dried spotted knapweed flower heads, containing *Urophora affinis*, were placed within the test plot to allow for natural eclosion and attack of the developing spotted knapweed flower buds.

Spotted knapweed plants growing with a grass treatment were significantly smaller ($P = 0.00$), while phosphorous fertilization resulted in significantly higher spotted knapweed dry weight ($P = 0.01$). However, significantly more *U. affinis* galls per seed head were found in the control without phosphorous, suggesting smaller, less vigorous plants may be more susceptible to attack by *U. affinis*. These results also suggest the importance of evaluating fertilization impact on both the target weed and biocontrol agents before such activities are widely adopted.

Using Koch's postulates, a bacterial pathogen *Pseudomonas syringae* pv *syringae* van Hall was isolated from diseased spotted knapweed stem and bud tissue in the field project. Usually considered an epiphyte, it is suggested that the bacterium took advantage of environmental conditions, a cold and wet season, and high levels of bud wounding from the artificially inflated *U. affinis* population.

1. INTRODUCTION

Spotted knapweed, *Centaurea maculosa* Lam., is considered by many to be the most serious weed in Montana. Most likely introduced in the 1900's as a contaminant in alfalfa seed, spotted knapweed has infested an estimated 4.7 million acres of range and pastureland in Montana (Lacey 1989); and is present in at least 34 states and Canada. It is estimated that over 7.25 million acres are heavily infested with spotted knapweed (Lacey et al. 1995).

With its low palatability to domestic livestock and wildlife and its invasive characteristics, spotted knapweed can reduce the carrying capacity of native rangeland by 90 percent (Bucher 1984). At the present rate of invasion, the potential annual loss to Montana's range livestock industry could reach $155 million dollars (Bucher 1984).

Spotted knapweed's ability to invade disturbed soils and its persistence once established require an integrated weed management plan. Control strategies include a combination of biological, chemical, and cultural methods (Lacey et al. 1995). Management plans should be long term and tailored for the size and location of the spotted knapweed infestation.

One of the first insect biological control agents introduced against spotted knapweed is the seed head fly, *Urophora affinis* Faurenfeld. It was introduced in 1973 and is successfully established in Washington, Oregon, Wyoming and Montana (Story 1978). The female fly deposits eggs within the developing knapweed flower bud and gall tissue subsequently surrounds the developing larvae. Flies develop and overwinter within the seed head and emerge as adults the following year. Plants with galls have fewer seeds (Harris 1980).

One of the most important factors affecting biocontrol of weeds programs is the presence of plant competition. While insect agents usually do not eradicate the target weed population, often they can slow growth or reproduction to a level that allows native vegetation to compete and reclaim lost territory (Huffaker and Messenger 1976). Fertilization and watering have been explored as possible cultural control practices that can enhance native forb and grass plant competition (Lacey et al. 1995).

During the 1980's, spotted knapweed was believed to be allelopathic, releasing chemical compounds into the soil that hindered germination and growth of beneficial plants (Kelsey and Locken 1987; Locken and Kelsey 1987). However, Harvey and Nowierski (1989) failed to demonstrate allelopathy in a greenhouse study using a number of grass and forb species growing in soil previously infested with spotted knapweed. In this study, knapweed infested soil contained significantly lower levels of nitrogen, phosphorous, and potassium compared to non-knapweed soil from the same site. This suggested nutrient depletion may play a role in spotted knapweed's competitive dominance in many plant communities.

The purpose of this study was to: 1) quantify the utilization of nutrients and water by spotted knapweed and two grass competitors; and 2) evaluate the effects of fertilization, plant competition, and the seed head fly, *U. affinis*, on the growth and reproduction of spotted knapweed.

2. LITERATURE REVIEW

Centaurea maculosa Lam.

Taxonomy

Centaurea maculosa

Division Tracheophyta

Class Angiospermae

Family Asteraceae

Centaurea maculosa Lamarck

Range and Ecology

Spotted knapweed was introduced from Eurasia in contaminated alfalfa and clover seed around 1893 (Lacey et al. 1995). It was first reported in Montana in the mid-1920's and by 1982, it was reported in every county (Forcella and Harvey 1981). Scattered knapweed populations are present in at least 34 states and Canada. It is estimated that over 7.25 million acres in these areas are heavily infested with spotted knapweed (Lacey 1989).

Requisites for spotted knapweed growth were identified in a study by Chicoine et al. (1989) Results of the study predicted where knapweed will grow based on soil type, annual precipitation, number of frost free days, elevation, potential evapotranspiration, and mean maximum July temperature. Composite maps were overlaid and showed that 37 million acres of Montana were vulnerable to spotted knapweed invasion (Chicoine et al. 1989).

Spotted knapweed is a member of the family Asteraceae. It is classified as a biennial or short-lived perennial forb; although there have been reports of individual knapweed plants living up to twelve years, with taproots large enough to count the annual xylem rings (Boggs and Story 1987). Knapweed is quick to establish on disturbed soil, and their early spring growth makes them competitive for soil moisture and nutrients (Whitson 1992).

Seedlings overwinter as a rosette. Plants can have one or more branched stems that bolt in June. Flowers are usually pinkish-purple and the flowering period extends from June to October. Each plant can produce 400-25,000 seeds depending on moisture level. The seeds, or achenes, are about 1/8 inch long and tipped with a tuft (Whitson 1992), and can stay dormant in the soil for eight to ten years (Davis et al. 1993). High seed production coupled with seed dormancy results in a well stocked and persistent seed bank.

Economic Importance

Spotted knapweed infests more than 4.5 million acres of rangeland and ranks as the number one noxious weed problem in western Montana (Lacey 1989). Kelsey and Mihalovich (1987) examined the nutrient composition of spotted knapweed and concluded that knapweed fiber, carbohydrate, and protein content has some nutritional value as a livestock forage. However, this invader is considered to have a low palatability to domestic livestock and wildlife and can reduce the carrying capacity of native rangeland by 90 percent (Bucher 1984). Given the right conditions, spotted knapweed overwhelms native vegetation, severely affecting wildlife habitat and livestock ranges. With the present rate of invasion, the potential annual loss to Montana's range livestock industry could reach $155 million dollars (Bucher 1984).

Soil and water resources are also adversely influenced by spotted knapweed. Simulated rainfall condition yield 56 percent higher runoff and 192 percent higher sediment yield on spotted knapweed sites versus bunch grass dominated sites (Lacey et al. 1989).

Control Strategies

Spotted knapweed's ability to invade disturbed soils and its persistence once established require an integrated weed management plan. Management plans should be long term and tailored for the size and location of knapweed infestation.

Chemical control can be effective for small patches of spotted knapweed and should be applied to the rosette stage of invading plants. Tordon is the most effective herbicide for controlling spotted knapweed, and is applied when plants are sending up a seedstalk (Lacey et al. 1995). However, chemical control is often cost prohibitive, especially on lower value rangeland.

Biological control of weeds uses natural enemies, insects, predators and pathogens, to curtail plant growth or reproduction. Biological control is environmentally safe, selective, economical, self perpetuating and can be incorporated into integrated management plans (Huffaker and Messenger 1976).

Twelve insects, all native to Eurasia, have been introduced into Montana as biological control agents (Montana Biological Weed Control Committee - 4/96). These include: four seed head flies *(Urophora affinis* Faurenfeld, *Urophora quadrifasciata* Meigen, *Chaetorellia acrolophi* White, and *Terellia virens* Loew), a seed head attacking moth *(Metzneria paucipunctella* Zeller), three root moths *(Agapeta zoegana* L., *Pelochrista medullana* Stgr., and *Pterolonche inspersa* Stgr.), a root weevil *(Cyphocleonus achates* Fahr.), and three seed head weevils (*Bangasternus fausti* Reitter, *Larinus minutus* Gyll., and *Larinus obtusus* Gyll.).

In addition to the insects there are also two fungal pathogens being investigated as biological control agents. They are both indigenous to Montana and include a crown fungus *Sclerotinia sclerotiorium* de Bary and a root fungus *Fusarium avenaceum* Sacc. (Montana Biological Weed Control Committee - 4/96). There has also been a report of a rust pathogen *Puccinia jacea* var. *diffusa* infecting diffuse knapweed, *Centaurea diffusa* Lam. (Palm et al. 1992).

Cultural control practices include hand pulling, grazing, mowing, cultivation, and irrigation. Hand pulling must be repeated annually until the seed bank has been exhausted (Lacey et al. 1995). Studies have shown that spotted knapweed occurs in a range of habitats from full sunlight to shady sites (Kennett et al. 1992). Knapweed invasion along roadsides can be reduced by not harvesting roadside timber which provides shade and poorer conditions for weed growth (Losensky 1989).

Motorized vehicles are a primary source of knapweed seed transport (Trunkle and Fay 1991) and, because of this, should not be driven through heavy knapweed infestations. Spotted knapweed seeds were also found to contain elaiosomes, indicating that this species is probably myrmechorous, or dispersed by ants (Pemberton and Irving 1990).

It is possible to combine different control strategies to produce an integrated management plan. Timing is crucial when combining chemical and biological control. Knapweed should be sprayed at the appropriate stage to maximize chemical impact, and to minimized interference on biocontrol agent growth and reproduction (Lacey et al. 1995).

Maxwell et al. (1992) explored the effects of grazing, spraying, and seeding on diffuse knapweed (*Centaurea diffusa* Lam.) in British Columbia. Knapweed cover was significantly lower on sprayed plots, however seeding with crested wheatgrass (*Agropyron cristatum* L.) resulted in the lowest knapweed coverage on unsprayed plots. Grazing pressure increased disturbance and resulted in a surge of knapweed growth.

With its invasive capabilities, extensive taproot system and ability to produce a large, persistent seed bank (Davis et al. 1993;Lacey et al. 1995), spotted knapweed management plans must be integrated and last for several years. Future control strategies will probably incorporate a cumulative approach, combining stress imposed by biocontrol agents and increased competition by the other vegetation through grazing regimes and reseeding programs (Muller-Scharer and Schroeder 1993).

Urophora affinis Faurenfeld

Taxonomy

Urophora affinis

Class Insecta (=Class Hexapoda)

Order Diptera

Family Tephritidae

Urophora affinis Faurenfeld

Range and Ecology

Urophora affinis is a Eurasian seed head fly. It was introduced into Montana as a biological control agent of spotted knapweed in 1973 (Story and Anderson 1978). The fly is now widely distributed and established in knapweed infested areas throughout the Pacific Northwest including: British Colombia, California, Idaho, Oregon, Washington, Wyoming and Montana (Story et al. 1989b).

Females oviposit in developing knapweed buds, and gall formation is subsequently produced. Besides occupying space in the seed head, galls create a metabolic sink that reduces seed production in attacked and unattacked flower head on the knapweed plant (Harris 1980).

At a typical population level (about two galls/seed head), studies have shown that *Urophora affinis* can reduce spotted knapweed seed production by at least 40 percent (Story et al. 1989b). Total seed reduction at fly-infested sites may exceed this amount due to long term, cumulative effects. However, *U. affinis* impact by itself will not be enough to control spotted knapweed. While seed reduction is an important first step toward biological control of spotted knapweed, other agents and management strategies will be necessary to prevent the spread and reduce present knapweed infestations (Story et al. 1989b).

Pseudomonas spp.

Taxonomy

Pseudomonas syringae

Division Schizomycetes

Order Eubacteriales

Family Pseudomonadacea

Pseudomonas syringae van Hall

Bacteria in the genus *Pseudomonas* are Gram-negative, non-sporing, long rods, motile with polar flagella, and weak fermenters of carbon compounds; they use nitrate as a nitrogen source (Dowson 1957). Pseudomonads are usually white when seen in mass on solid media and motile when young. Most species fluoresce diffusible pigments, forming creamy growths that can later turn purple (Holt et al. 1994). Several pseudomonads are phytopathogenic. Disease symptoms for this genus often cause a disintegration of parenchyma, and while infection generally takes place through the stomata, leaf scars and wounds also provide places of entry (Dowson 1957).

Micro-organisms are reported to winter on or in normal dormant buds of some perennial plants. The bud as a source of epiphytic microflora may be called the 'gemmisphere' (Leben and Lange 1971). Growing buds with free moisture provide a favorable environment for micro-organisms.

Pseudomonas syringae, *Pseudomonas cichorii*, and *Pseudomonas fluorescens* have all been associated with a disease described as stem pith necrosis in tomato plants (Martins 1989). Disease symptoms include laddering or complete disintegration of pith tissues and dark patches on the stem surfaces. When vascular tissues are invaded the plant wilts. These bacteria are usually considered epiphytes due to their dependence on exceptional disease enhancing factors, such as excessive moisture, nutritional imbalances, and cold temperatures (Martins 1989).

Hydroponics

Hydroponics is the growth of plants in a nutrient solution. The requirements for plant growth include: radiant energy, water, carbon dioxide, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, molybdenum, chlorine, boron, and copper (Jutras 1979). Nutrient solutions provide water, and macro- and micronutrients necessary for plant growth. With proper care, plants grown in nutrient solutions can give equal or higher yields per unit of area, compared to plants grown in highly productive soils (Schippers 1986).

There have been many studies that use hydroponics to monitor water and nutrient requirements of plants (Pettersson et al. 1993; Gutschick and Kay 1991; Alt and Struwe 1982; Menn and McBee 1970). The benefits include relatively easy manipulation of nutrient levels that can be monitored to help determine limiting factors and nutrient requirements for maximum yield.

Biological Control

In his book, An Introduction to Biological Control, Robert van den Bosch writes that, 'Biological control is a natural phenomenon- the regulation of plant and animal numbers by natural enemies.' Most of the noxious weeds in the United States were introduced from foreign countries. Usually, these exotic plants arrive at their new location without natural enemies, and can reproduce and spread more successfully than in their native country (Rees et al. 1996).

Classical biological control involves the collection of natural enemies of the target weed from its place of origin and their importation and release into the area where the weed has become a problem. The desired effect is a reduction in growth, reproduction and spread of the weed to allow native and other more desirable vegetation to compete (Huffaker and Messenger 1976).

Limiting Factors and Plant Competition

Barbour et al. (1980) defined competition as the mutually adverse effects of organisms (plants) which use a resource in short supply. These resources can include light, water, and nutrients. Competition for water is often the most variable resource necessary for plant growth (Radosevich and Holt 1984). In rangeland conditions, competition for water can be extremely fierce. There is also a strong interaction between water usage and nutrient availability. Root development may require adequate nutrition and have a direct impact on a plants ability to compete for water (Radosevich and Holt 1984).

Plant species growing in proximity can compete for nutrients. Weeds generally have a well developed, extensive root system which makes them strong competitors for water and nutrients. The elimination of such weeds should provide increased productivity of desirable plants (Radosevich and Holt 1984).

Reader and Watt (1981) applied nitrogen, phosphorous, and potassium fertilizer to an abandoned pasture and found an increase in the grass standing crop, a reduction in the hawkweed (*Hieracium floribundum* Wimm. and Grab.) crop, and a temporary halt of hawkweed patch formation. This suggested soil fertility could influence the outcome of grass-hawkweed interactions in the field.

However, increased soil fertility does not always translate into reduced weed density (Story et al. 1989a). This may be due to luxury consumption by the weed (Vengris et al. 1955), or an increase in soil fertility may translate to increased weed growth with increased resource utilization (Radosevich and Holt 1984).

Bacterial Identification

Bacteria can be identified using morphological characters, such as colony shape, arrangement, position of flagella, and position of spores or physiological characters, such as differences in their biochemical activities and antigenic structure

(Dowson 1957). Two methods for bacteria identification discussed in this paper are Gas Chromatography Fatty Acid Methyl Ester (GC-FAME) and Biolog™ carbon source test.

Bacteria have a unique fatty acid composition and it is possible to identify bacteria based on their fatty acid content using the GC-FAME system. Fatty acids are extracted from a pure culture of bacterium. These fatty acids are injected into a gas chromatograph and a chromatogram is formed of the fatty acid composition. The chromatogram is matched to other fatty acid profiles in the data base and the resulting match identifies the bacterium (MiL, Inc. 1996).

Different bacteria can use different combinations of carbon sources for growth. The Biolog™ system of identification uses a 96-well microtiter plate containing 95 different carbon sources. If the bacterium can grow on a carbon source a dye in the well turns purple. Resulting purple patterns are read by an automated microplate reader and compared to patterns in the data base. The match identifies the bacterium (MiL, Inc. 1996).

3. STUDY I: WATER AND NUTRIENT UPTAKE OF SPOTTED KNAPWEED AND TWO GRASS COMPETITORS IN A HYDROPONIC SYSTEM

Objective and Hypothesis

The objective of the greenhouse study was to quantify water, nitrogen, phosphorous, and potassium uptake in spotted knapweed, *Centaurea maculosa* Lam., and two grass competitors: western wheatgrass, *Pascopyrum smithii* (Rydb.) Love, and a crested wheatgrass hybrid, *Agropyron cristatum* (L.) Gaert X *Agropyron desertorum* (Fisch. ex Link) Schult., using a hydroponic system..

The corresponding hypothesis is that differences in water and nutrient uptake exist for spotted knapweed, western wheatgrass, and crested wheatgrass.

Materials and Methods

Hydroponic Drip System

The frame for the hydroponic drip system was constructed out of 1" X 2" fir stripping. Plywood was laid down, and forty 6" diameter holes were cut out with a jigsaw to hold pots in place. Forty, two-liter pop bottles were collected, washed with a mixture of soap and bleach, spray painted white to reduce light penetration, and attached to the frame using wire run through their plastic bases. Pop lids were fit with Fisherbrand^R 1000 microliter disposable pipet tips. A 20 cm length of 3/8" surgical tubing connected each two-liter bottle with an individual potted plant.

Forty, 150 X 180 mm pots were filled with two layers of 76 X 150 mm Grodan[R] HP inert rock wool growing media. A 150-mm round disc of 5 mm black plastic was laid on top of the media to reduce algal growth. Plastic lids, each 15 cm in diameter, were snapped on top of the pots to reduce evaporation. A 4 cm hole was centered in the lid to allow for plant growth. Each of the forty pots were positioned in a 150 X 180 mm white, plastic bucket fit to catch and recycle the nutrient solution.

Seeds of spotted knapweed (n=108), Rosana western wheatgrass (n=98), and Hycrest crested wheatgrass (n=96) were germinated in rock wool and distilled water on 27 September 1994. Spotted knapweed seed was collected in the Bison Range, MT on 11 September 1990, while seed of the two grass species was obtained from USDA Soil Conservation Service: Bridger, Montana.

Each of the forty pots containing dry media were weighed individually and 2 liters of nutrient solution were added to each on 20 October 1994. The nutrient solution was mixed in bulk using the recipe in Table 18 (see Appendix).

Ten seedlings of knapweed, western, and crested wheatgrass were transplanted from the germinating tray to individual pots in the drip system. Plants were placed in a complete randomized block design, with ten blocks. Each block contained four pots: a knapweed treatment, a western wheatgrass treatment, a crested wheatgrass treatment, and a control with no plant. The greenhouse was set at 23° C with 14 hour days.

On each sampling date 140 ml of nutrient solution was collected from each treatment and analyzed for nitrogen (NO3-N), phosphorous, and potassium concentrations (mg/L) by the Soil Analytical Lab at Montana State University. Nitrate concentrations were tested using the automated cadmium reduction method, while phosphorous and potassium concentrations were tested using Inductively Coupled Plasma (ICP) technique (Clesceri et al. 1989). On each sampling occasion, each pot and catch bucket was weighed, using a Sartorius[R] 2500 g scale, to estimate water loss. Excess nutrient solution was then dumped from the catch bucket and all treatments received 2 liters of new nutrient solution. Fresh solution was also tested each time for nitrogen, phosphorous, and potassium concentrations to ensure consistency in nutrient levels.

Plant material was harvested and weighed, at the conclusion of the experiment. Leaves were stripped from the plants and run through a LI-Cor[R] LI-3100 Area Meter, twice. Plant material from each plant was then placed in brown paper bags and baked in an oven (37 °C, 20 K/Pa) for 30 days, and weighed. Pots containing media and plant root material were also baked in an oven (37°C, 20 k/Pa) for 30 days, and weighed. Starting pot dry weights were subtracted from final pot dry weights to estimate root biomass. Control pot difference (Mean = 8.48 g., S.E. = 0.300) was subtracted from treatments to compensate for any remaining water or concentrated salts.

Statistical Analysis

Statistical analysis was performed by MSUSTAT, version 5.20. Analysis of Variance (ANOVA), multiple comparisons (COMPARE), and multiple regression (MREGRESS) subroutines were used. Fitted treatment means with multiple comparisons were computed using Newman-Keuls (Sequential Studentized Range) (Snedecor and Cochran 1980). Graphs were produced using SigmaPlot. T-tests were conducted using MATHCAD.

Results

Hydroponic Study

One spotted knapweed plant did not survive transplant. This accounts for a sample size ($n = 9$) for spotted knapweed, while the grasses and control pots each had a sample size ($n = 10$).

Plant Biomass and Leaf Area

The dry weight of spotted knapweed above the hydroponic media exceeded that of western wheatgrass and crested wheatgrass by 258 and 50 %, respectively (Table 1, $P < 0.01$). The lowest above-media dry weights were obtained for western wheatgrass, with a mean weight that was 58% lower than that obtained for crested wheatgrass.

Despite having lower above media biomass, the leaf area of crested wheatgrass exceeded that of spotted knapweed and western wheatgrass by 28 and

297%, respectively (Table 2, $P < 0.01$). Western wheatgrass had the lowest leaf area, with a mean that was 210% lower than that obtained for spotted knapweed.

Similarly, crested wheatgrass had higher root biomass than spotted knapweed and western wheatgrass (Table 3, $P <= 0.01$). Root biomass in crested wheatgrass exceeded that in spotted knapweed and western wheatgrass by 30.6 and 126%, respectively.

As a consequence, spotted knapweed had a significantly lower root:shoot biomass (ratio 0.717; Table 4, $P = 0.03$), compared to that of crested wheatgrass and western wheatgrass. This suggests that spotted knapweed allocates more resources to above media vegetation in this system.

Water Uptake

Cumulative rate of water uptake was significantly higher for spotted knapweed throughout the hydroponic experiment (Figure 1). Crested wheatgrass used the second highest amount of cumulative water, followed by western wheatgrass. Cumulative water lost in the control pots was low (final mean = 762 ml $\pm$ S.E. 21.32). This is less than 5% of the total amount of solution poured into the system, suggesting water loss due to evaporation was small.

Rates of water uptake (ml/day) during each sampling interval were also higher for spotted knapweed during the first 100 days of the experiment (Figure 2). Differences were largest during the period between day 46 and day 88. These intervals correspond to the development time when eight of the nine knapweed plants were bolting.

As expected, spotted knapweed had a significantly higher water use-efficiency ratio (WUE) than either crested or western wheatgrass (ratio 2.13, Table 5, $P = 0.03$). Crested wheatgrass had a slightly higher WUE than western wheatgrass, but the difference was not significant ($P > 0.05$).

Nutrient concentrations remaining at each sampling date were compared with cumulative water uptake using linear regression analysis (Figure 3, 4, 5). Starting concentrations of the nutrients and their concentrations at each of the eight sampling dates comprised the nine points used in the analyses. Nutrient concentrations remaining are assumed to be a reflection of plant uptake and water use.

Results in Table 6 and Table 7 show that spotted knapweed and western wheatgrass have significantly flatter slopes for nitrogen and potassium levels when compared to crested wheatgrass. This suggests that spotted knapweed and western wheatgrass plants absorb more nitrogen and potassium with each ml of water than crested wheatgrass, in this system.

Conversely, results in Table 8 show crested wheatgrass to have a significantly flatter slope for phosphorous concentrations when compared to spotted knapweed and western wheatgrass, suggesting that crested wheatgrass absorbs phosphorous more readily than the other two plant species.

Table 1: Dry Weights of above-media plant material obtained in the hydroponic study

Treatment	Dry Weight (g) Mean	± S.E.		N
Western Wheatgrass	8.914	1.06	A	10
Crested Wheatgrass	21.26	2.60	B	10
Spotted Knapweed	31.90	2.12	C	9

$P < 0.01$

Table 2: Final leaf area obtained in the hydroponic study

Treatment	Leaf Area (cm^2) Mean	± S.E.		N
Western Wheatgrass	355	52.84	A	10
Spotted Knapweed	1099	244.20	B	9
Crested Wheatgrass	1410	103.97	B	10

$P < 0.01$

Table 3: Root biomass estimates obtained in the hydroponic study

Treatment	Root Mass Estimates (g) Mean	± S.E.		N
Western Wheatgrass	13.55	1.71	A	10
Spotted Knapweed	23.46	2.07	B	9
Crested Wheatgrass	30.63	3.22	C	10

$P < 0.01$

Table 4: Plant root:shoot biomass ratios obtained in the hydroponic study

Treatment	Root Dry Weight (g)/Shoot Dry Weight (g) Mean	± S.E.		N
Spotted Knapweed	0.717	0.109	A	9
Crested Wheatgrass	1.513	0.116	B	10
Western Wheatgrass	1.964	0.474	B	10

P = 0.03

Table 5: Plant water use efficiency ratios (WUE) obtained in the hydroponic study

Treatment	Stem Dry Weight(g)/Cumwater (L) Mean	± S.E.		N
Western Wheatgrass	1.41	0.294	A	10
Crested Wheatgrass	1.54	0.094	A	10
Spotted Knapweed	2.13	0.072	B	9

P = 0.03

FIGURE 1: Cumulative water used among three plant species over time (Hydroponic Study I)

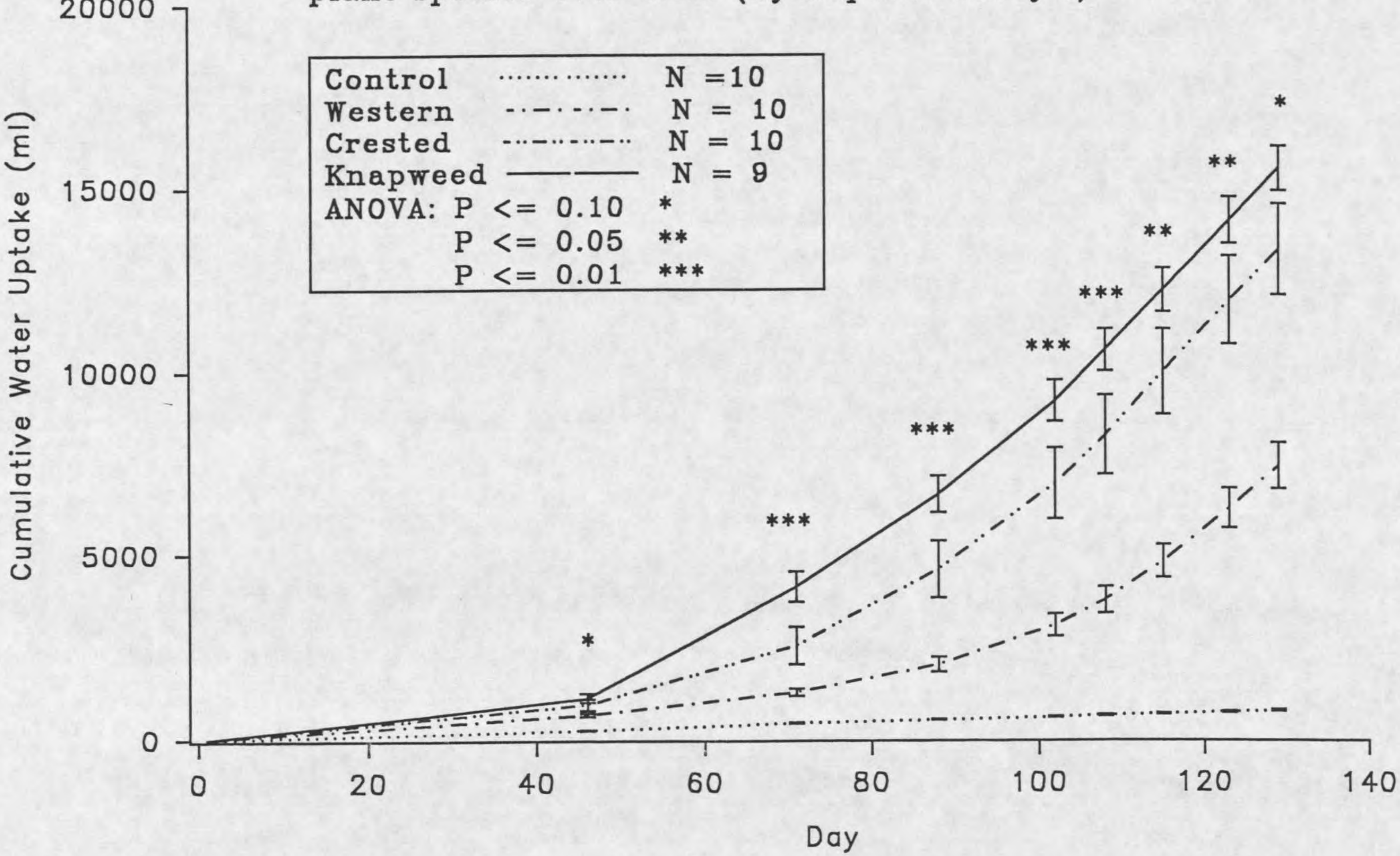

FIGURE 2: Water rate used during each interval among three plant species over time (Hydroponic Study I)