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
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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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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

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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.

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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)

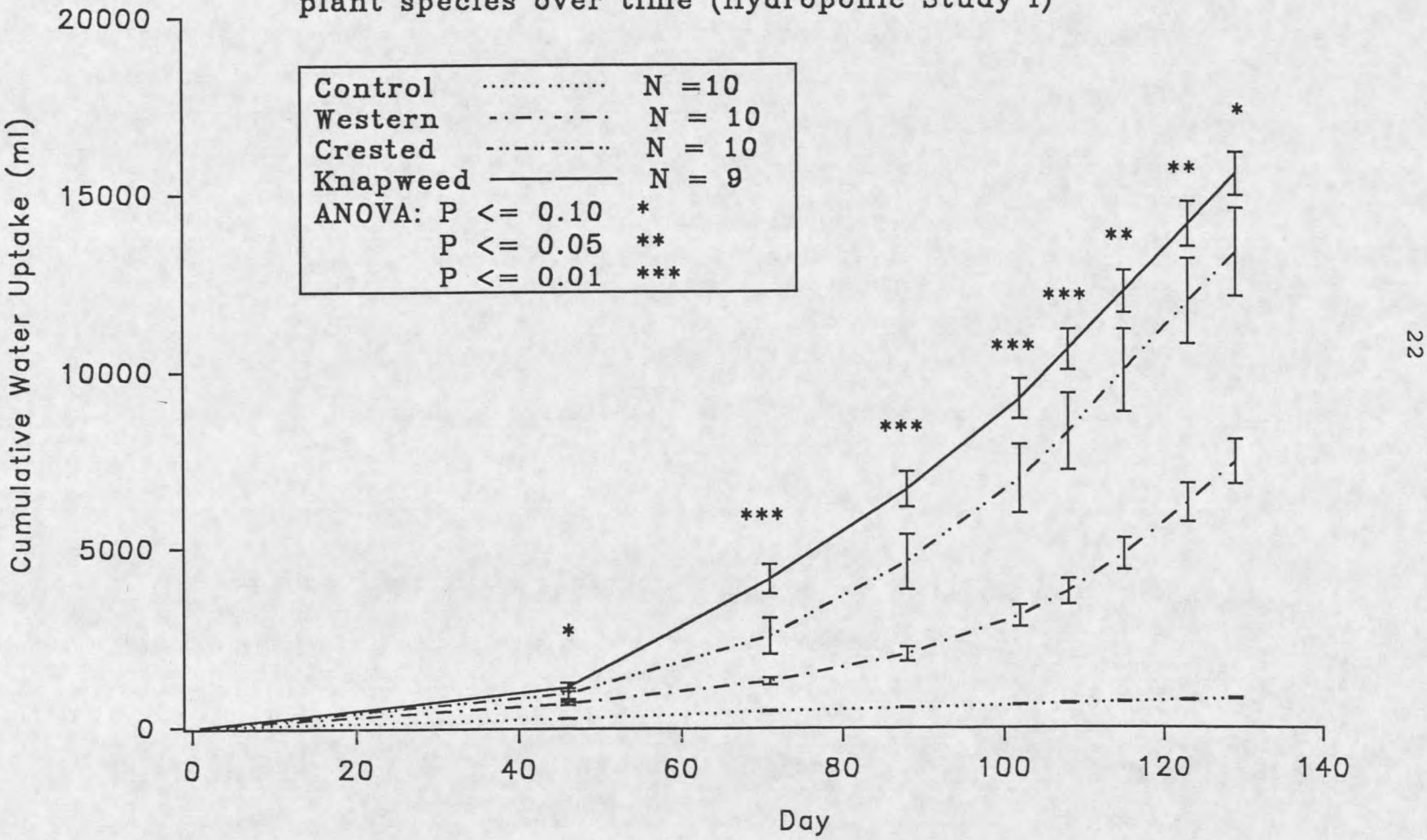


FIGURE 2: Water rate used during each interval among three plant species over time (Hydroponic Study I)

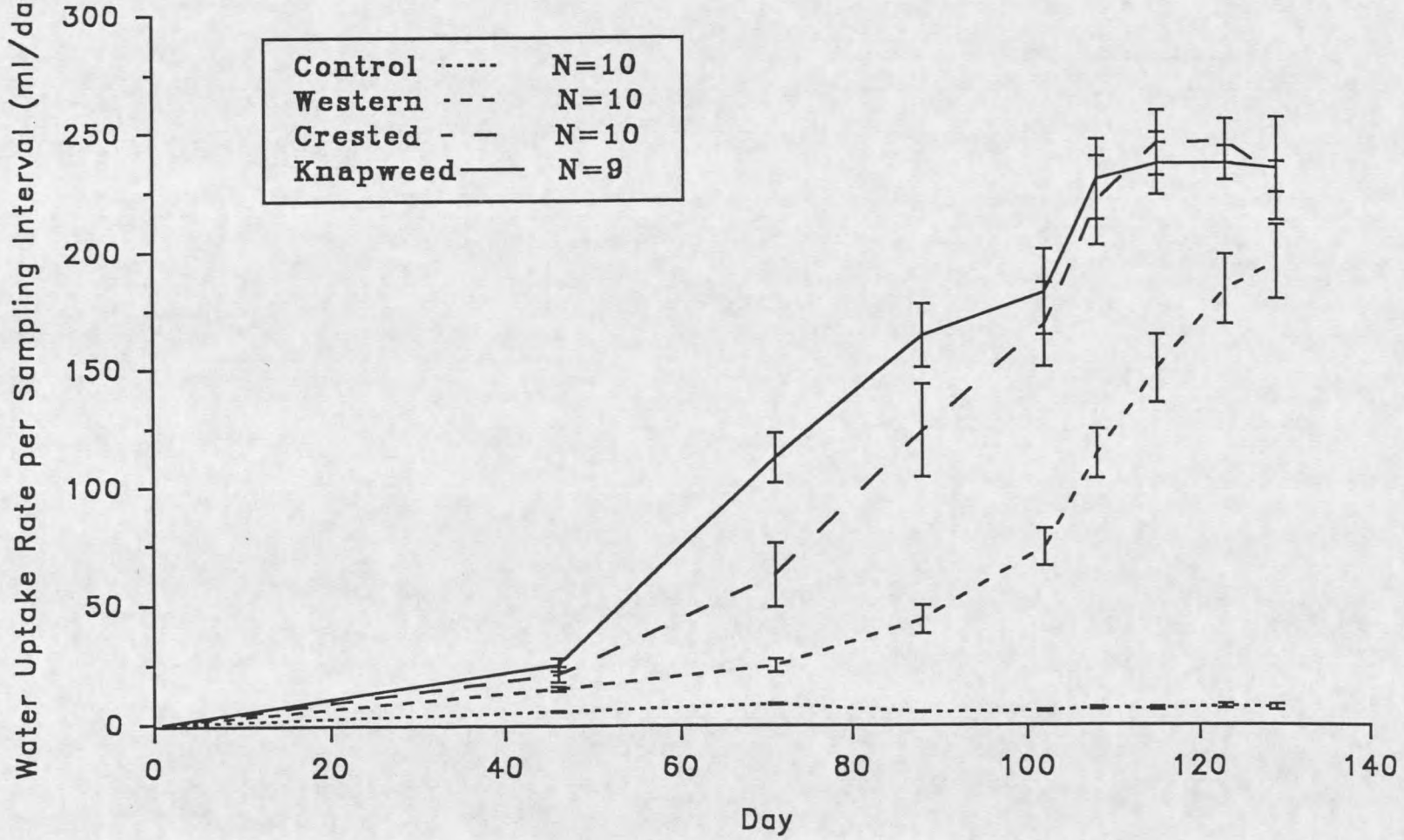


FIGURE 3: Regression relationship between mean nitrogen remaining and cumulative water used (Hydroponic Study I)

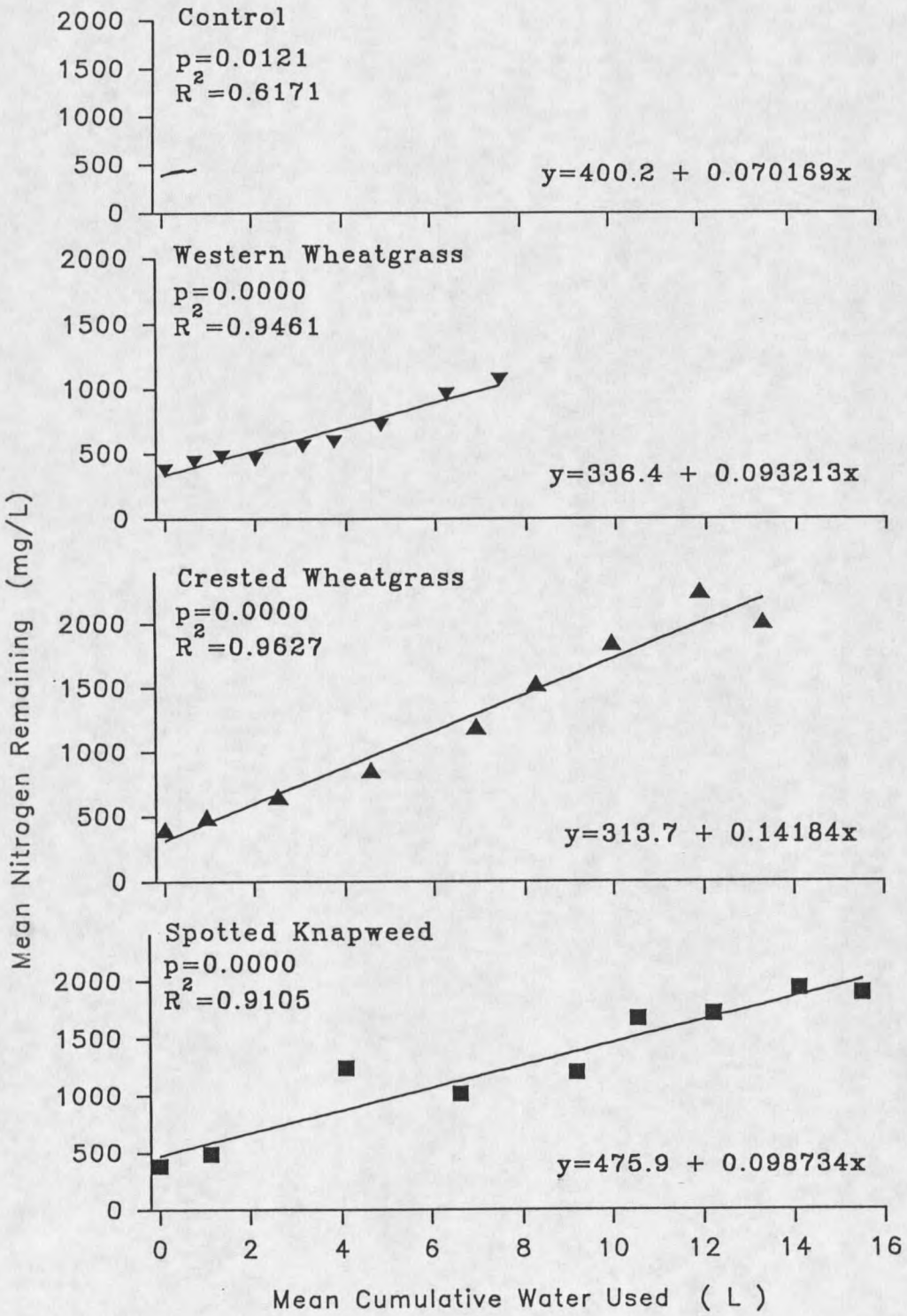


FIGURE 4: Regression relationship between mean potassium remaining and cumulative water used (Hydroponic Study I)

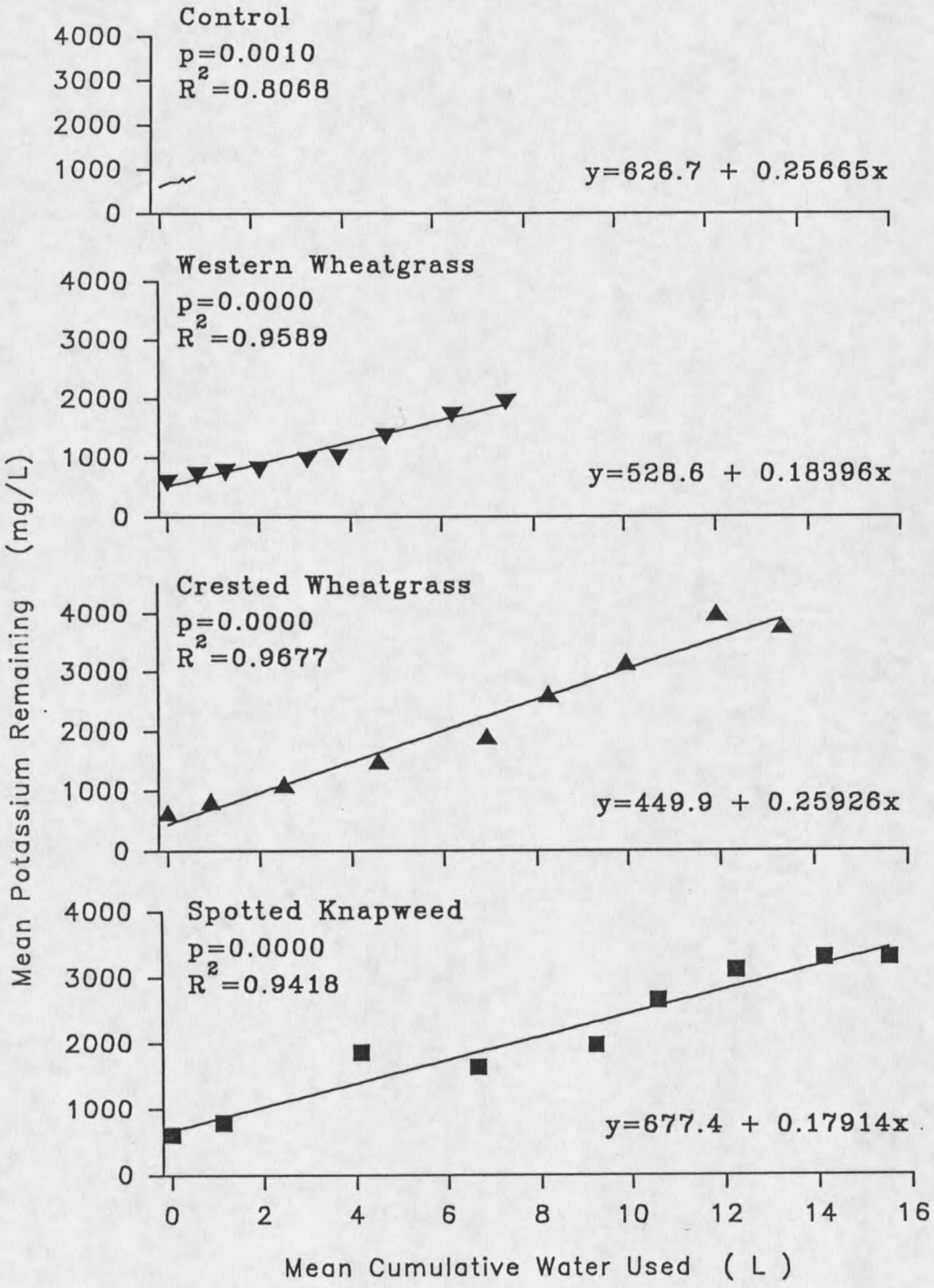


FIGURE 5: Regression relationship between mean phosphorous remaining and cumulative water used (Hydroponic Study I)

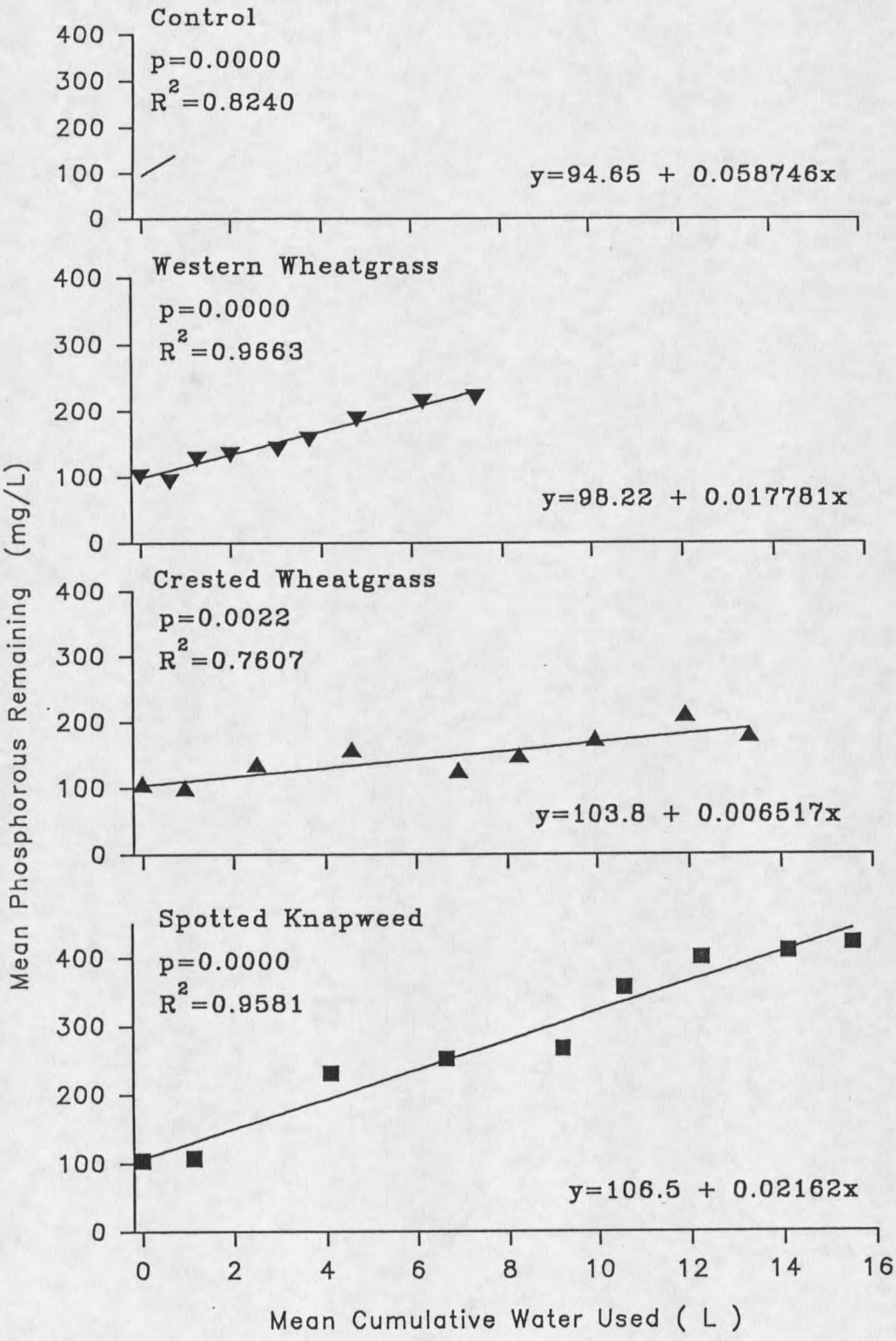


Table 6: Slope comparison test of the regression of mean nitrogen remaining against cumulative water use by plants in the hydroponic study

Treatment	b	±S.E.	T-value	P-value
Spotted knapweed	0.0987	0.012		
Western Wheatgrass	0.0932	0.008	0.389	>0.50
Crested Wheatgrass	0.1418	0.011	-2.736	0.025
Control	0.0702	0.021	1.193	0.400
Western Wheatgrass	0.0932	0.008		
Crested Wheatgrass	0.1418	0.011	-2.763	0.025
Control	0.0702	0.021	1.02	0.400
Crested Wheatgrass	0.1418	0.011		
Control	0.0702	0.021	3.062	0.010

Degrees of Freedom = 14

Table 7: Slope comparison test of the regression of mean potassium remaining against cumulative water use by plants in the hydroponic study

Treatment	b	±S.E.	T-value	P-value
Spotted knapweed	0.1791	0.017		
Western Wheatgrass	0.1840	0.014	0.218	>0.50
Crested Wheatgrass	0.2593	0.018	-3.259	0.010
Control	0.2567	0.047	-1.539	0.200
Western Wheatgrass	0.1840	0.014		
Crested Wheatgrass	0.2593	0.018	-3.278	0.010
Control	0.2567	0.047	-1.465	0.200
Crested Wheatgrass	0.2593	0.018		
Control	0.2567	0.047	0.051	>0.50

Degrees of Freedom = 14

Table 8: Slope comparison test of the regression of mean phosphorous remaining against cumulative water use by plants in the hydroponic study

Treatment	b	±S.E.	T-value	P-value
Spotted knapweed	0.0216	0.0017		
Western Wheatgrass	0.0178	0.0013	1.812	0.100
Crested Wheatgrass	0.0065	0.0014	6.876	0.001
Control	0.0587	0.0103	-3.569	0.001
Western Wheatgrass	0.0178	0.0013		
Crested Wheatgrass	0.0065	0.0014	6.036	0.001
Control	0.0587	0.0103	-3.963	0.001
Crested Wheatgrass	0.0065	0.0014		
Control	0.0587	0.0103	-5.045	0.001

Degrees of Freedom = 14

Discussion

Hydroponic Study

The hydroponic system provided an artificial environment to track water and nutrient uptake rates for spotted knapweed, and western and crested wheatgrass. It is important to remember that plants can respond very differently to environmental factors in the field and when grown together versus individually (Williams 1963). Although comparisons between very different plant types, spotted knapweed (a dicot) and grasses (monocots) have been likened to comparing apples and oranges, some of the conclusions drawn from this system are supported by results obtained in field studies.

Water evapotranspiration

Spotted knapweed used significantly more cumulative water throughout the hydroponic experiment than either crested wheatgrass or western wheatgrass. The rate of water use may change with plant development, and this appeared to be the case with spotted knapweed as differences were largest during bolting.

Competition for water can be especially fierce in arid rangeland conditions, where water supply cannot by supplemented by irrigation (Radosevich and Holt 1984). Hence, plants in such situations that have the capacity for rapid and early root growth should maintain their competitive advantage throughout the growing season (Pavlychenko 1937).

The results obtained in the leaf area studies are somewhat ambiguous as, measurements were not taken until the end of the experiment, and by this time many of the large rosette leaves of spotted knapweed had dried up. Desiccated and brittle leaves were found to break into tiny pieces, stick to the leaf area meter and could not be measured for area. Unfortunately, the sample size of the experiment did not allow for destructive sampling to measure leaf area throughout plant development. However, crested wheatgrass and knapweed's larger leaf area explain their ability to use more water than the western wheatgrass and control treatments.

Spotted knapweed's higher water use efficiency ratios are another indication of its ability to outcompete grass competitors for water and to survive water stress. However, WUE ratios do not always explain competitive ability. Pearcy et al. (1981) showed that differences in WUE ratios between common lambsquarter (*Chenopodium album*) and redroot pigweed (*Amaranthus retroflexus*) did not affect competitive ability for water. The ability to survive water stress and ability to outcompete a neighboring plant for a limited resource are not necessarily the same phenomenon (Radosevich and Holt 1984).

Frank (1994) compared physiological differences between crested wheatgrass and western wheatgrass with respect to water. His analysis of later stages of grass development found western wheatgrass to be more tolerant of water stress under field conditions. Western wheatgrass' lower values for water uptake throughout the hydroponic experiment are most likely a function of its lower dry weight and slower development.

The amount of water available for use under a competitive system depends on the plant species in the system. "Water users" are able to control the amount of water available by evapotranspiring large amounts of water, while "water conservers" sacrifice productivity to survive in a water limited environment (Radosevich and Holt 1984). It appears that relative to one another knapweed and crested wheatgrass apply a user strategy, while western wheatgrass uses a more conservative strategy.

Nutrient uptake

Measurements of nutrient samples reflect concentrations remaining in the system. It is assumed that these concentrations are a result of plant use, evaporation, and transpiration. With this in mind, the results of regression analysis were interpreted as follow: a flatter slope translates to more nutrients appropriated per unit of water.

Nitrogen and potassium have very different mobility in comparison with phosphorous. Nitrogen and potassium are considered to have high mobility because they dissolve in soil water. In contrast, phosphorous is considered to have low mobility, often being rapidly fixed to the soil (Teng and Timmer 1995). In the hydroponic system, it is assumed that all nutrients are dissolved in solution and are unrestrictedly available. Nutrient mobility may account for spotted knapweed nutrient acquisition. In a soil media, nutrients would behave differently.

Plants are thought to absorb mineral elements indiscriminately through their rooting medium (Radosevich and Holt 1984). Vengris et al. (1955) compared

nutrient levels of corn grown with and without weeds. He found nitrogen and potassium levels of corn were significantly lower in plants grown with weeds. Story et al. (1989a) compared effects of nitrogen fertilization on a site composed of knapweed and crested wheatgrass. In that study, crested wheatgrass did not respond to fertilization treatments, while knapweed did show significant response to nitrogen treatment in the first year. Harvey and Nowierski (1989) proposed nutrient depletion to help explain knapweed's domination of certain plant communities. Spotted knapweed's ability to absorb water, nitrogen, and potassium more efficiently than crested wheatgrass tend to support the nutrient depletion hypothesis.

Different nitrogen and potassium rates for western wheatgrass and crested wheatgrass suggest different mechanisms for nutrient uptake between the two grass species. Gray et al. (1953) found differential potassium uptake between grass and legume species was correlated with root cation exchange capacities. The results of regression analysis in this study suggest western wheatgrass is more competitive for nitrogen and potassium resources.

Lower slopes for crested wheatgrass phosphorous absorption suggest plants do not absorb minerals indiscriminately. Harvey and Nowierski 1989 proposed that spotted knapweed competes by removing phosphorous from the soil at an accelerated rate or by converting phosphorous to an unavailable form. The results from regression analysis showed knapweed to have a larger slope than both western and crested wheatgrass, suggesting a slower mechanism for phosphorous uptake.

Spotted knapweed has a stout taproot (Whitson et al. 1992), while the grass plants have a fibrous root system. van Noordwijk and Willigen (1991) described relationships between root characteristics and nutrient uptake. They concluded that for nutrients with low mobility, like phosphorous, high root length density is the main requirement. For nutrients of high mobility, such as nitrogen and potassium, a greater rooting depth is required to obtain a high efficiency of nutrient uptake. Differences in root morphology may help to explain nutrient adsorption trends in the hydroponic system.

Petterson et al. (1993) conducted a study to examine the growth and nutrient uptake of spring barley under different water and nutrient regimes. Results showed drought conditions decreased growth and the uptake of phosphorous substantially, and they concluded the effect of drought on crop growth could have been due to inadequate phosphate uptake. It is conceivable that results from Harvey and Nowierski (1989) were a function of spotted knapweed's ability to deplete water resources, making phosphate less available for neighboring vegetation.

4. STUDY II: GRASS, PHOSPHOROUS, AND SEED HEAD FLY EFFECT ON SPOTTED KNAPWEED UNDER FIELD CONDITIONS

Objective and Hypothesis

The objective of the field study was to examine a combination of phosphorous and grass treatments in addition to a seed head fly biological control agent, *Urophora affinis*, and their effects on spotted knapweed growth and reproduction.

The corresponding hypothesis is that a combination of grass interference, phosphorous treatment and infestation with the seed head fly *Urophora affinis* will provide maximum interference on spotted knapweed growth and reproduction.

Materials and Methods

Field Study

Spotted knapweed seed was planted in the center of 120, 46 cm X 20 cm, tapered tree pots on 4 June 1994. Spotted knapweed received a combination of a grass treatment (1 Rosana western wheatgrass plant, 1 Hycrest crested wheatgrass plant, or control solitary knapweed plant with no grass) and a phosphorous treatment (0.5 liter at 60 mg/L, 120 mg/L, 240 mg/L, or control distilled water). The pots were arranged in a complete randomized block design with 10 blocks, each containing 12 treatments: solitary spotted knapweed plant (no P, 60 mg/L, 120

mg/L, 240 mg/L), single spotted knapweed with single western wheatgrass (no P, 60 mg/L, 120 mg/L, 240 mg/L), and single spotted knapweed growing with single crested wheatgrass (no P, 60 mg/L, 120 mg/L, 240 mg/L).

Grass treatment pots were planted with 5 seeds Rosana western wheatgrass, or 5 seeds Hycrest crested wheatgrass. Knapweed seed was collected on the Bison Range, MT on 11 September 1990. Grass seed was from USDA Soil Conservation Service: Bridger, MT. Pots were thinned to one spotted knapweed plant and one grass plant per pot.

Phosphorous treatments were applied at monthly intervals. Half a liter of complete general fertilizer, minus phosphorous, was applied to all treatments at the same time as phosphorous treatment.

Post holes, each approximated 25 cm wide by 60 cm deep, were dug on campus at Montana State University and the tree pots were positioned in each hole and buried on 12 July 1994. Pots were arranged in a complete randomized block design. Pots were positioned so the grass treatment pointed south to provide maximum interference for light. During the first month, plants were replaced with back up seedlings from flats and watered as necessary to ensure early survival.

During the second field season, bouquets of dried knapweed seed heads containing *U. affinis* flies were collected from Story Hills, Bozeman MT on 24 May 1995. Initial seed head fly density were 2.64 galls/ head. Using temperature data from Sept 1994 through April 1995, fly mortality was estimated at approximately 50%. Estimating for 50 flies per knapweed plant, stems with approximately 100

buds were staked on the NW corner of each pot on 26 May 1995. Flies eclosed naturally and stung developing buds of spotted knapweed.

Above ground plant material was harvested from pots on 10 November 1995. Knapweed and grass plants were put into brown paper bags and oven dried (37 ° C, 20 k/Pa) for sixty days, and plant material was weighed. Then, a random sample of knapweed seed heads from different treatments was collected and dissected to count the number of *U. affinis* galls per seed head present.

Results

Field study spotted knapweed biomass

A multi-factorial analysis of variance was performed on the combinations of grass and phosphorous treatments (Table 9). Comparisons were made on combinations of phosphorous and grass interference treatments (Table 10, $P < 0.01$).

The above ground dry weight was largest for spotted knapweed plants growing without a grass competitor (Table 10, $P < 0.01$). A comparison of grass treatment showed spotted knapweed plants growing by themselves were 75 and 113% larger than their counterparts growing with a crested wheatgrass and western wheatgrass plant, respectively (Table 11, $P < 0.01$). Although spotted knapweed plants growing with western wheatgrass plants were 22% smaller than spotted knapweed growing with crested wheatgrass, this difference was not significant ($P > 0.10$).

Similarly, above ground dry weight was higher in spotted knapweed plants that received a phosphorous treatment (Table 10, $P < 0.01$). When comparisons were made on phosphorous treatment only, spotted knapweed growing without any phosphorous fertilizer where at least 44% smaller than their counterparts that received some level of phosphorous (Table 12, $P = 0.0021$). No significant difference was observed between levels of phosphorous fertilization (Table 12, $P > 0.10$).

Field study grass biomass

Phosphorous treatment did not appear to affect grass biomass (ANOVA, $P = 0.87$). Direct comparisons of all grass treatments growing with spotted knapweed showed western wheatgrass to have 72% larger above ground dry weight than crested wheatgrass plants (Table 13, $P < 0.01$). This value incorporates 12 crested wheatgrass plants that did not survive until harvest, and 1 western wheatgrass plant that did not survive. When comparing dry weights of grass plants that survived, western wheatgrass was only 23% larger than crested wheatgrass (western wheatgrass- mean = 15.51 g $\pm$ S.E. = 1.30, crested wheatgrass - mean = 12.57 g $\pm$ S.E. = 1.42).

Field Study Seed Head Fly Density

It is assumed that *Urophora affinis* had equal access to all spotted knapweed plants in the field experiment. Presence of a grass treatment did not appear to affect

U. affinis gall density of spotted knapweed in the field project (ANOVA, $P = 0.3687$). Spotted knapweed samples were grouped by levels of phosphorous fertilization, and plants that received a phosphorous treatment had significantly fewer galls / seed head than control plants that received no phosphorous (Table 14, $P = 0.01$). Although spotted knapweed plants receiving higher levels of phosphorous contained fewer galls per seed head than plants with lower levels of phosphorous, the difference was not significant ($P > 0.10$).

Table 9: Multi factorial ANOVA of phosphorous and grass interference effect on spotted knapweed dry weight obtained in the field study.

Source	S.S.	M.S.	p-value	D.F.
Block	4128.8	458.75		9
Phosphorous	3548.3	1182.8	0.0021	3
Grass Interference	13687	6843.3	0.0000	2
Phosphorous * Grass	3581.7	596.95	0.0197	6
Residual	22246	224.71		99

Table 10: Dry weights of spotted knapweed with and without grass interference in combination with various phosphorous treatments obtained in the field study.

Treatment Grass (Phosphorous)	Spotted Knapweed Dry Weight (g) Means	± S.E.		N
Western (Control)	10.47	2.85	A	10
Western (60 mg/L)	21.85	5.05	ABC	10
Western (120 mg/L)	21.27	4.48	ABC	10
Western (240 mg/L)	33.27	7.40	BC	10
Crested (Control)	23.21	3.27	ABC	10
Crested (60 mg/L)	20.77	4.19	AB	10
Crested (120 mg/L)	34.77	6.44	BC	10
Crested (240 mg/L)	27.21	7.10	BC	10
Knapweed only (Control)	34.13	2.32	BC	10
Knapweed only (60 mg/L)	58.36	5.58	E	10
Knapweed only (120 mg/L)	41.68	3.82	CD	10
Knapweed only (240 mg/L)	51.35	3.63	DE	10

P-value < 0.01

Table 11: Dry weights of spotted knapweed with and without competition from grasses obtained in the field study.

Treatment Grass Interference	Spotted Knapweed Dry Weight (g) Mean	±S.E.		N
Western Wheatgrass	21.72	2.816	A	40
Crested Wheatgrass	26.49	2.765	A	40
Control (Knapweed alone)	46.38	2.429	B	40
P-value < 0.01				

Table 12: Dry weights of spotted knapweed with various phosphorous treatments obtained in the field study.

Treatment Phosphorous	Spotted Knapweed Dry Weight (g) Means	± S.E.		N
Control	22.61	2.394	A	30
Low (60 mg/L)	33.66	4.267	B	30
Medium (120 mg/L)	32.57	3.219	B	30
High (240 mg/L)	37.28	3.984	B	30
P-value < 0.01				

Table 13: Dry weights of grass species obtained in the field study.

Treatment	Grass Dry Weight (g) Mean	± S.E.		N
Crested Wheatgrass	8.80	1.350	A	40
Western Wheatgrass	15.12	1.301	B	40
P-value < 0.01				

Table 14: Density of *Urophora affinis* galls / seed head under various phosphorous treatments (Field Study).

Treatment Phosphorous	*U. affinis* galls (# / seed head) Mean	± S.E.		N
High (240 mg/L)	5.26	0.471	A	90
Medium (120 mg/L)	6.01	0.385	A	90
Low (60 mg/L)	6.27	0.478	A	90
Control	7.39	0.498	B	90
P-value = 0.01				

Discussion

Field Study Knapweed Biomass

It was not surprising that knapweed plants growing without a grass treatment had larger dry weights. With no neighboring plant to compete for water and nutrients, knapweed plants were able to grow larger. Kennet et al. (1992) found similar results with spotted knapweed root and crown growth being adversely affected by increasing competition from bluebunch wheatgrass, *Pseudoroegneria spicata.* With hindsight, it would have been more appropriate to have two knapweed plants growing together as a control to test intra- versus interspecific plant interactions.

Knapweed plants receiving phosphorous fertilizer were larger than control plants. Story et al. (1989a) found a positive response to knapweed growth when nitrogen fertilizer was applied in a field situation. The results from this field study show similar trends with phosphorous fertilizer. It appears that by itself, fertilizing spotted knapweed with nitrogen or phosphorous results only in larger spotted knapweed plants. This suggests reduction of a spotted knapweed stand by other methods, biological, chemical or cultural control, would be a prudent first step before increasing soil fertility.

Grass Biomass

Phosphorous treatment did not seem to directly affect either grass dry weight, however phosphorous fertilization resulted in a positive response in above ground dry weights of spotted knapweed plants. The results from STUDY I suggested western and crested wheatgrass were more efficient at phosphorous adsorption. The increased dry weight of spotted knapweed plants with added phosphorous seem to support conclusions from the hydroponic system.

Western wheatgrass and crested wheatgrass are cool-season grasses used for grazing throughout the intermountain west (Frank and Bauer 1991). Crested wheatgrass is considered an early grass, producing abundant early spring forage, but little summer regrowth. Conversely, western wheatgrass generally produces less spring forage than crested wheatgrass, but production generally continues throughout the growing season. These two different growth patterns effect the response of these species to available soil water supply (Frank and Bauer 1991).

Frank and Bauer (1991) conducted a study examining rooting activity and water use during vegetative development of crested and western wheatgrass. They found that water use-efficiency (WUE) of crested wheatgrass decreased with increased water treatment and development, while western wheatgrass WUE did not change with either. In 1994, Frank found western wheatgrass to be more tolerant of water stress than crested wheatgrass under field conditions.

Western wheatgrass' different development pattern, coupled with a consistent water use-efficiency throughout development, and its more efficient nutrient uptake

rates (STUDY I), provide possible explanations for its larger above ground dry weight than crested wheatgrass.

Frank et al. (1990) also compared the effects of soil water, nitrogen and growing degree days on morphological development of crested and western wheatgrass. They found that plants develop primarily in response to air temperature and not added water or nitrogen. Frank et al. (1990) found that initial foliage growth of crested wheatgrass and western wheatgrass required 82 and 98 growing degree-days (GDD), respectively; while regrowth foliage of crested wheatgrass and western wheatgrass required 372 and 135 more GDD, respectively.

This suggests that crested wheatgrass develops faster initially, but western wheatgrass is faster at producing regrowth foliage. These trends were seen in the greenhouse(STUDY I) and field study (STUDY II). Crested wheatgrass growing by itself in a hydroponic system, developed faster and produced larger plants. Western wheatgrass growing with spotted knapweed produced larger plants after two years in the field study .

Seed Head Fly Density

Control knapweed plants that did not receive phosphorous fertilizer had more *U. affinis* galls. There are several possibilities for this trend. *Urophora affinis* has been shown to prefer a narrow range of smaller knapweed buds (Story et al. 1989b). Control plants had a smaller biomass, and at the time of oviposition may have provided a greater number of smaller buds acceptable for oviposition.

Another explanation is the possible occurrence of different levels of plant defense between fertilization treatments. There have been many studies investigating fertility effects on plant defense (Gershenzon 1994; Stockhoff 1994; Felton and Summers 1993). Fertilized plants may have produce buds that were biochemically or physiologically less attractive. Further studies are required to determine *U. affinis* bud selection with respect to spotted knapweed nutrition.

A few knapweed stems were observed with signs of predation. Story et al. (1995) reported predation on fly galls by three predator/herbivores: the deer mouse, *Peromyscus maniculatus* (Wagner); the black capped chickadee, *Parus atricapillus* L.; and the white-tailed deer, *Odocoileus virginianus* (Zimmermann). No active predation was witnessed, but chew marks on infested buds similar to those found in the study by Story et al. (1995), suggested seed head predation by small rodents.

5. STUDY III: ISOLATION, IDENTIFICATION, AND IMPACT OF BACTERIAL PATHOGENS (*PSEUDOMONAS* SPP.) AFFECTING SPOTTED KNAPWEED IN THE FIELD.

Objective and Hypothesis

The objective of this study was to isolate the pathogen responsible for widespread disease symptoms of spotted knapweed plants in the field project (STUDY II).

The corresponding hypothesis is that the disease observed in the field project was caused by the bacterium *Pseudomonas syringae* pv. *syringae.*

Materials and Methods

Bacterial isolation

Bacteria were isolated from diseased spotted knapweed stems using Koch's postulates (Dowson 1957). Knapweed buds and stems showing disease symptoms were harvested from the field project (figure 6, 7, 8). Symptoms included curling of spotted knapweed stems at the tip similar to a shepherd's crook. The outside surface of the knapweed stems were brown and covered with saprophytes. Usually a constriction was present between diseased and healthy stem tissue. Dissection of the stem showed disintegrated pith, while infected vascular tissue was black.

Plant tissue was surface sterilized with a 10% Clorox solution for three minutes, rinsed in double distilled, sterilized water and mechanically crushed with a mortar and pestle. Crushed tissue was then plated onto Modified King's B media described in Table 19 (see Appendix). Isolates were stored in sterile water and plated onto slants of Modified King's B media for identification.

A tobacco hypersensitivity test was conducted with two field isolates. Two day old bacteria cultures were mixed with 0.1 molar K buffer. Using a SPECTRONIC 20 spectrometer, solutions were mixed at absorptivity = 0.260, wavelength 640 nm. Bacterial solutions from the field were then injected into tobacco leaves. Buffer was injected into tobacco leaves as a control and a known pathogen *Pseudomonas tagetis* was injected as a positive control. Leaves were examined 24 hours later to check for cell collapse.

Two day old bacteria cultures that showed signs of pathogenicity in the tobacco hypersensitivity test were mixed on a petri dish with 9 ml of 0.1 molar K buffer. The bacterial solution [log 10.43] was mixed with a surfactant, 0.2% silwet, and aerosol sprayed onto a healthy knapweed plant. Sterile water mixed with 0.2% silwet was used as a check. A second treatment involved injecting 1 ml of the bacteria solution [log 10.34] into healthy, developing spotted knapweed buds. Sterile water (1 ml) was injected as a check.

Bacteria were isolated from the injection treatment knapweed stems using the same protocol described above. Field isolates and greenhouse re-isolates were sent for identification using GC-FAME and Biolog™ processing (see Appendix:Figure 9).

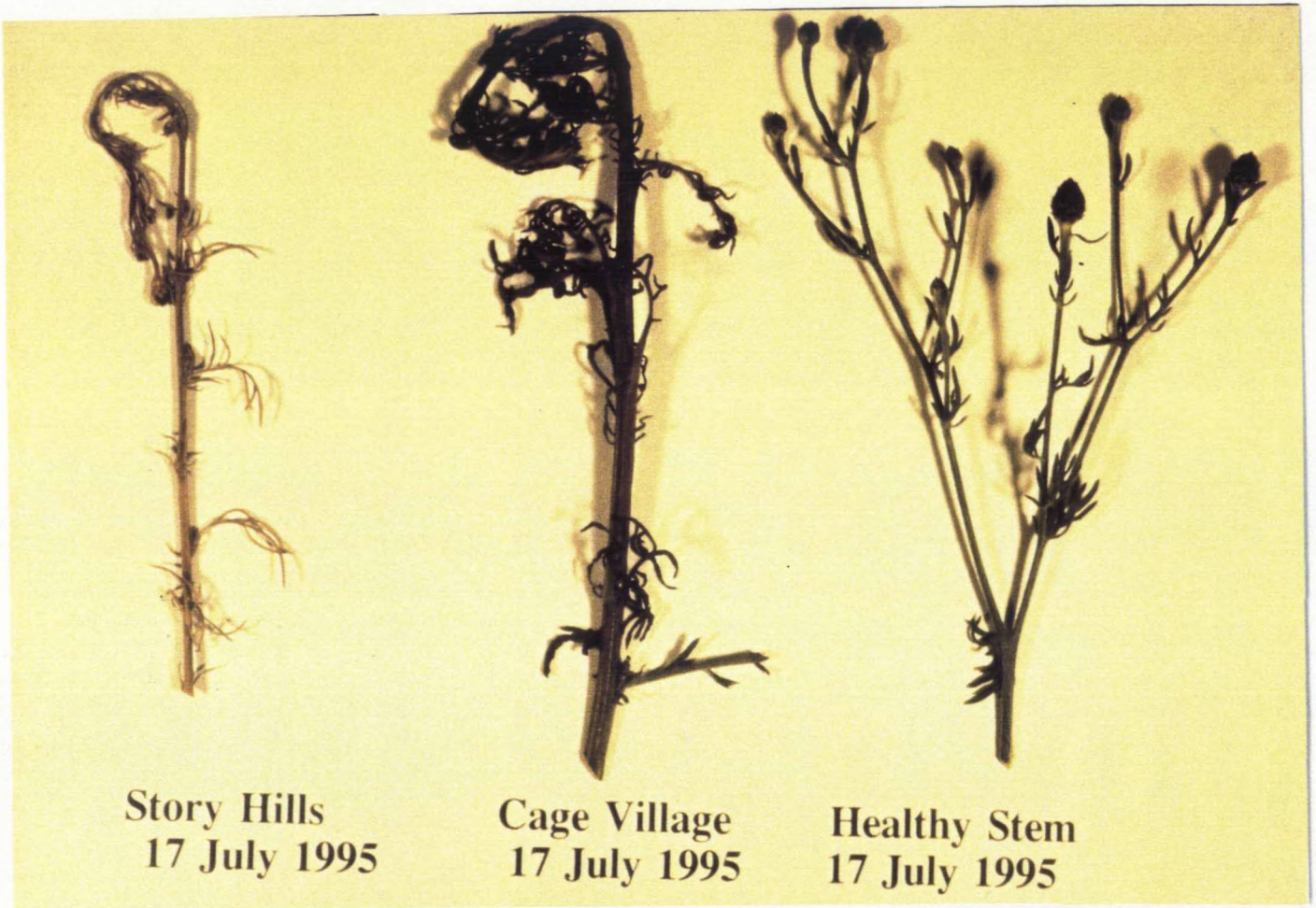


Figure 6: Spotted knapweed stems from Story Hills, MT and field study (STUDY II) Bozeman, MT showing disease symptoms.

Figure 7: Disease symptoms in spotted knapweed stems from Field Study II

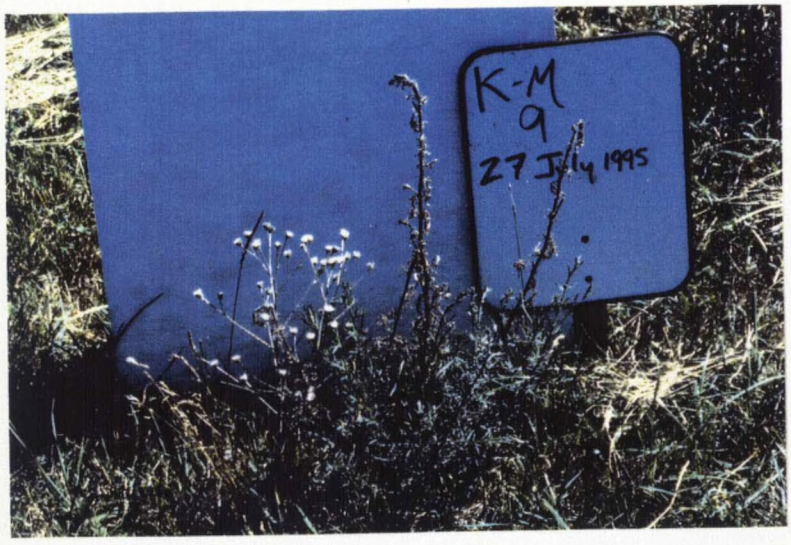


Figure 8: Close up disease symptoms in spotted knapweed stems from Field Study II.



Results

Bacteria Isolation, Identification, and Impact

More than 36% of spotted knapweed plants growing in the field project (STUDY II) showed disease symptoms. Infection of spotted knapweed plants appeared to be random. Above ground dry weight of spotted knapweed was 67% larger for healthy plants compared to plants showing presence of wilt symptoms (Table 15, $P < 0.01$).

After 24 hours, the known pathogen, *Pseudomonas tagetis*, and one strain from the field study showed cell collapse in the tobacco hypersensitivity test. The sterile water control and other field isolate failed to show pathogenic symptoms. The field pathogen was identified as *Pseudomonas syringae* pv *syringae* (GC-FAME results) and *Pseudomonas syringae* pv *aptata* (Biolog™ results) (Table 16).

Bacteria isolates were collected from spotted knapweed plants showing similar wilt symptoms seen in STUDY II, at three other field locations in Montana. These were also injected into developing spotted knapweed buds and re-isolated from stems that later showed symptoms. Field isolates and re-isolates were identified by GC-FAME (Table 17). Two spotted knapweed plants with symptoms were placed in the Montana State University Herbarium, Voucher S. Kearing No. 1 and Voucher S. Kearing No. 2.

Table 15: Dry weight of spotted knapweed with and without disease symptoms (Field Study II).

Wilt Symptoms	Spotted Knapweed Dry Weight (g) Mean	± S.E.		N
Present	22.09	2.130	A	44
Absent	36.99	2.385	B	76
P-value < 0.01				

Table 16: Identification of pathogenic bacterium isolated from spotted knapweed plants with disease symptoms.

Technique	ID	Similarity Coefficient	Distance Coefficient
GC-FAME	*Pseudomonas syringae* pv *syringae*	0.945	1.191
Biolog™	*Pseudomonas syringae* pv *aptata*	0.840	1.522

Table 17: Bacteria isolated from spotted knapweed plants with symptoms from three field locations in Montana.

Technique	Location	ID
GC-FAME	MSU Ag Research Farm Bozeman, MT	*Pseudomonas syringae*
GC-FAME	Story Hills Bozeman, MT	*Pseudomonas cichorii*
GC-FAME	Rock Creek exit I-90 Rock Creek, MT	*Pseudomonas flourescens*

Discussion

Bacterial Isolation, Identification and Impact

Disease results from interactions involving the plant, the pathogen, and the environment including moisture, temperature, and nutrients (Huber 1994). Whether a disease is severe or latent depends on the interactions of these factors.

Spotted knapweed plants showed symptoms during stem elongation and bud development only. Symptoms appeared to move from tip down the stem. In most cases, an infected stem was not fatal to the plant, and the spotted knapweed plant was able to compensate by developing uninfected stems.

Presence of disease symptoms in STUDY II resulted in smaller spotted knapweed plants. This was an unplanned component that attacked knapweed indiscriminately, that may have compromised results of planned treatments. I only recorded presence/ absence of disease symptoms. A more in depth study would be required to estimate the actual impact of the wilt on the growth and reproduction of spotted knapweed.

The tobacco hypersensitivity test provided evidence that the bacterium isolated from STUDY II was pathogenic. Re-isolation of the bacterium from injected spotted knapweed buds showing similar disease symptoms support the bacterial pathogen hypothesis. This is the first documentation of the bacterial pathogen *Pseudomonas syringae* on spotted knapweed.

No disease symptoms were seen when the bacterial mixture was sprayed on with a surfactant. Injecting into bud tissue was necessary to see wilt symptoms in greenhouse plant. This suggests a wound is necessary for bacterial infection.

Pseudomonads are usually dispersed by rain splash (Dowson 1957). Several pseudomonads are phytopathogenic. Disease symptoms often cause a disintegration of the parenchyma, and while infection generally takes place through the stomata, leaf scars and wounds also provide places of entry (Dowson 1957). Growing buds with free moisture provide a favorable environment for micro-organisms (Leben and Lang 1971).

Pseudomonas syringae, *Pseudomonas cichorii*, and *Pseudomonas flourescens* 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 surface. When vascular tissues are invaded, the plant wilts. These bacteria are usually considered epiphytes due to their dependence on disease enhancing factors such as excessive moisture, nutritional imbalances and cold temperatures (Martins 1989).

Climatological data from the National Oceanic and Atmospheric Administration (NOAA) showed the Montana State University station to have below normal temperature readings for May, June, and July 1995. A artificially inflated *Urophora affinis* population infested buds at a much higher rate (5-7 galls/seed head) than normal field conditions (2 galls/seed head)(Story et al. 1989b). Cold temperatures, high rates of bud wounding by *U. affinis*, and excess water provided

ideal environmental conditions for the bacterium to infect the spotted knapweed plants in STUDY II.

Pseudomonads isolated from three field locations in Montana did not show the high rates of infection seen in STUDY II. Diseased plants had a very sporadic distribution, and were usually in higher than normal moisture areas. The low levels of spotted knapweed with disease symptoms seen in the field suggest cold and wet environmental conditions, in addition to a seed head fly creating a bud wound, are necessary to achieve infection with *Pseudomonas* spp. under field conditions.

6. SUMMARY

Spotted knapweed is considered by many to be the most serious rangeland weed in western Montana. A hydroponics study conducted at Montana State University, Bozeman MT found spotted knapweed to use significantly more water than Rosana western wheatgrass or Hycrest crested wheatgrass. Regression analysis showed spotted knapweed and western wheatgrass to absorb nitrogen and potassium more efficiently than crested wheatgrass. Conversely, the crested and western wheatgrass were shown to absorb phosphorous at faster rates. These results suggest that spotted knapweed may gain a competitive edge by depleting water, nitrogen, and potassium resources.

A field study was conducted in Bozeman, MT to examine effects of phosphorous fertilization, plant competition, and infestation with the biological control agent *Urophora affinis* on spotted knapweed growth and reproduction. Spotted knapweed growing with a western wheatgrass or crested wheatgrass plant were significantly smaller than spotted knapweed plants growing by themselves.

Fertilization with phosphorous resulted in larger spotted knapweed plants, but had no effect on grass biomass. Phosphorous treatment also resulted in lower numbers *U. affinis* galls / seed head, suggesting smaller, less vigorous spotted knapweed plants may be more susceptible to attack by *U. affinis*. Results from the field study reveal the importance of evaluating increased soil fertility and plant competition on both the target weed, neighboring vegetation, and biocontrol agent before such activities are widely adopted.

A previously undescribed plant pathogen was isolated from spotted knapweed plants in the field project. Using Koch's postulates, the bacterium *Pseudomonas syringae* pv *syringae* was isolated and identified as the pathogen. Usually considered an epiphyte, it is suggested that the bacterium took advantage of ideal environmental conditions, a cold and wet season, and high levels of bud wounding from the biological control agent *Urophora affinis*.

Spotted knapweed has been a focus of weed scientists in Montana for the last 20 years, resulting in an extensive data base. Given the right conditions, spotted knapweed has the ability to invade and become very successfully established. Much of spotted knapweed's competitive ability is a result of its root system.

Future management programs will need to integrate biological, chemical, and cultural control strategies. The combination of an insect biological control agent and a plant pathogen is a control strategy that warrants further investigation. Studies documenting spotted knapweed's root profile in the field, including resource allocation and use are also needed. A long term management plan will need to focus, at least partly, on the subterranean aspects of spotted knapweed.

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APPENDIX

Table 18:Hydroponic nutrient solutions used to study nitrogen, potassium and phosphorous utilization in spotted knapweed, western wheatgrass and crested wheatgrass.

Macronutrient solution		
g/L Distilled H_2O	Compound	
1.77	$Ca(NO_3)_2$* 4 H_2O	Calcium Nitrate Tetrahydrate
1.37	KNO_3	Potassium Nitrate
0.53	KH_2PO_4	Potassium Acid Phosphate
1.01	$MgSO_4$	Magnesium Sulfate
Micronutrient solution (add 1 ml/L to macronutrient solution)		
g/L Distilled H_2O	Compound	
2.86	H_3BO_6	Boric Acid
1.81	$MnCl_3$ * 4 H_2O	Manganese Chloride
0.22	$ZnSO_4$ * 7 H_2O	Zinc Sulfate
0.08	$CuSO_4$ * 5 H_20	Cupric Sulfate
0.01	H_2MoO_4 * 4 H_20	Molybdic acid

Table 19: Modified Kings B Media (Fluor. PS) used to isolate *Pseudomonas* spp. bacteria from infected spotted knapweed plants.

20.0 g	Proteose Peptone #3
1.5 g	K_2HPO_4* 3 H_2O
20 g	Agar
990 ml	Double Distilled Water
15 ml	Glycerol
1.5 g	$MgSO_4$ (anhy.)in solution
100.0 mg	Cycloheximide(2.5 ml/L)

$MgSO_4$ and Cycloheximide autoclaved separately

Figure 9: GC-FAME and Biolog™ processing used to identify strains of *Pseudomonas* spp. bacteria from infected spotted knapweed plants.

Procedure copied from MiL, Inc. Report No.: MILB-3799

Following isolation, the strains are individually streaked onto TSA. The strains are incubated for 24 hours and then processed by standard GC-FAME Method 1. The processed strains are examined against both the Aerobe (TSBA[rev. 3.90]) and Clinical Aerobe (CLIN [rev. 3.90]) GC FAME databases. Subsequently, the strains are prepared for Biolog™ analysis by suspending them in sterile saline and loading the saline solutions into the appropriate microtiter plates (Gram negative or Gram positive). The plates are incubated for 24 hours and then compared, using an automated microplate reader, to version 3.7 of the Biolog™ database.

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