Effects of defoliating leafy spurge on condensed tannin concentrations, sheep rumen microorganisms, and migratory grasshoppers
by Joanna L Roberts

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

Abstract:
Most herbivores avoid the noxious weed leafy spurge (Euphorbia esula L.) when grazing. However, some ruminants including sheep will consume leafy spurge, albeit only up to 50% of their total diet, possibly because of the presence of secondary compounds. Leafy spurge contains high concentrations of terpenoids. Additional secondary compounds may be present in the plant. Synergistic effects of terpenoids and other secondary compounds, including condensed tannins (CT), may determine the response of herbivores to leafy spurge. In addition, secondary compounds may increase in leafy spurge after herbivory.

Our first objective was to determine if CT, if present, increase in previously defoliated (PD) leafy spurge. Our second objective was to determine if material from undefoliated (U) or PD leafy spurge shoots adversely affects sheep rumen microorganisms and a generalist grasshopper (Melanoplus sanguinipes).

Effects of leafy spurge on sheep rumen microbial activity and mass were determined with in vitro dry matter disappearance (DMD) and microbial gas production. Undefoliated and PD leafy spurge was collected in June, July, and August 1994 from a leafy spurge-infested rangeland near Grass Range, Montana. Rumen microbial responses to 4 mixtures of leafy spurge leaves, flowers, and stems, and grass hay were analyzed. Effects of leafy spurge on weight and mortality of M sanguinipes nymphs were determined in 5-day feeding trials. Undefoliated and PD leafy spurge was collected in July and August 1995 from an infested site near Bozeman, Montana. Nymphs were fed 4 mixtures of leafy spurge plant parts with grass hay. All plant parts collected in 1994 and 1995 were analyzed for CT.

Condensed tannins were present in all leafy spurge plant parts collected in 1994 and 1995. Condensed tannin concentrations increased seasonally, and were the highest in stems from PD shoots. Increasing levels of leafy spurge leaves and flowers increased DMD, neutral detergent fiber disappearance (NDFD), and microbial mass. They also increased microbial activity up to the 75% leafy spurge level ($P<0.10$). In June, DMD, NDFD, microbial activity, and microbial mass were higher for leaves from PD shoots than for leaves from U shoots ($P<0.005$). In July, microbial activity and DMD were lower for stems from PD shoots than from undefoliated shoots ($P=0.0001$). Grasshopper nymphs consuming leaves and flowers weighed more and had lower mortality than those consuming stems ($P<0.0l$). Nymph weights were highest for nymphs consuming leaves from PD shoots ($P=0.0001$). Nymph weights were affected by the proportion of leafy spurge in mixtures for material collected in August only ($P<0.10$).

Sheep rumen microbes and M. sanguinipes nymphs were not adversely affected by leaves from PD leafy spurge shoots; defoliation did not increase CT concentrations of these leaves. In July, rumen microbial activity and DMD were lowest for stems from PD shoots; these responses correlate with seasonal increases in CT concentrations. Synergistic effects of CT and other secondary compounds in leafy spurge may determine the overall response of herbivores to the plant.

EFFECTS OF DEFOLIATING LEAFY SPURGE ON CONDENSED TANNIN CONCENTRATIONS, SHEEP RUMEN MICROORGANISMS, AND MIGRATORY GRASSHOPPERS

by

Joanna L. Roberts

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Range Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1996

APPROVAL

of a thesis submitted by

Joanna L. Roberts

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.

Bret E. Olson _____ 4/10/96
Date

Approved for the Department of Animal and Range Science

John Paterson _____ 4/10/96
Date

Approved for the College of Graduate Studies

Robert Brown _____ 4/20/96
Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under the rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Joanna L. Roberts

Date 4/11/96

Dedicated in memory of Dr. Verl M. Thomas.

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Bret Olson, and the other members of my graduate committee, Dr. Jan Bowman and Dr. Robert Nowierski, for their guidance and advice throughout my graduate program. In addition, I would like to thank Dr. Nancy Roth for sharing her ideas and assisting me with laboratory procedures, Rosie Wallander for assisting me with data analysis and graphics, and Jim Fischer for allowing the use of his equipment.

I would also like to thank all my friends and roommates for their moral support and invaluable assistance. Finally, I would especially like to thank my parents, my brother Ken, and Kristie Rausch for their tremendous support and encouragement, as well as the long and sometimes unusual hours they spent assisting me in the field and laboratory. Thank you for listening, understanding, and helping me stay focused on my goals.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

ABSTRACT

Most herbivores avoid the noxious weed leafy spurge (*Euphorbia esula* L.) when grazing. However, some ruminants including sheep will consume leafy spurge, albeit only up to 50% of their total diet, possibly because of the presence of secondary compounds. Leafy spurge contains high concentrations of terpenoids. Additional secondary compounds may be present in the plant. Synergistic effects of terpenoids and other secondary compounds, including condensed tannins (CT), may determine the response of herbivores to leafy spurge. In addition, secondary compounds may increase in leafy spurge after herbivory.

Our first objective was to determine if CT, if present, increase in previously defoliated (PD) leafy spurge. Our second objective was to determine if material from undefoliated (U) or PD leafy spurge shoots adversely affects sheep rumen microorganisms and a generalist grasshopper (*Melanoplus sanguinipes*).

Effects of leafy spurge on sheep rumen microbial activity and mass were determined with in vitro dry matter disappearance (DMD) and microbial gas production. Undefoliated and PD leafy spurge was collected in June, July, and August 1994 from a leafy spurge-infested rangeland near Grass Range, Montana. Rumen microbial responses to 4 mixtures of leafy spurge leaves, flowers, and stems, and grass hay were analyzed. Effects of leafy spurge on weight and mortality of *M. sanguinipes* nymphs were determined in 5-day feeding trials. Undefoliated and PD leafy spurge was collected in July and August 1995 from an infested site near Bozeman, Montana. Nymphs were fed 4 mixtures of leafy spurge plant parts with grass hay. All plant parts collected in 1994 and 1995 were analyzed for CT.

Condensed tannins were present in all leafy spurge plant parts collected in 1994 and 1995. Condensed tannin concentrations increased seasonally, and were the highest in stems from PD shoots. Increasing levels of leafy spurge leaves and flowers increased DMD, neutral detergent fiber disappearance (NDFD), and microbial mass. They also increased microbial activity up to the 75% leafy spurge level ($P<0.10$). In June, DMD, NDFD, microbial activity, and microbial mass were higher for leaves from PD shoots than for leaves from U shoots ($P<0.005$). In July, microbial activity and DMD were lower for stems from PD shoots than from undefoliated shoots ($P=0.0001$). Grasshopper nymphs consuming leaves and flowers weighed more and had lower mortality than those consuming stems ($P<0.01$). Nymph weights were highest for nymphs consuming leaves from PD shoots ($P=0.0001$). Nymph weights were affected by the proportion of leafy spurge in mixtures for material collected in August only ($P<0.10$).

Sheep rumen microbes and *M. sanguinipes* nymphs were not adversely affected by leaves from PD leafy spurge shoots; defoliation did not increase CT concentrations of these leaves. In July, rumen microbial activity and DMD were lowest for stems from PD shoots; these responses correlate with seasonal increases in CT concentrations. Synergistic effects of CT and other secondary compounds in leafy spurge may determine the overall response of herbivores to the plant.

CHAPTER 1

INTRODUCTION

Leafy spurge (*Euphorbia esula* L.) is an introduced perennial weed that infests millions of hectares in the Northern Great Plains (Lacey et al. 1985). This noxious weed causes economic losses to livestock producers by decreasing forage production and thus reducing rangeland carrying capacity (Reilly and Kaufman 1979). Chemical control of leafy spurge is expensive and is often not effective. Herbicides generally remove aboveground growth but do not fully penetrate the plant's massive root system (Lingle and Suttle 1985, Lym and Moxness 1989, Lym 1992), which produces many buds that produce new growth centers (Raju 1985). Biological control of leafy spurge is also difficult to accomplish because compounds in the plant's latex inhibit consumption by many herbivores (Lym and Kirby 1987). Cattle generally consume little, if any, leafy spurge when grazing (Lym et al. 1988). By selecting more desirable species on rangelands where leafy spurge is present, these animals increase the competitive advantage of leafy spurge over those species that are more desirable. However, domestic sheep have the potential to effectively control leafy spurge in Montana (Landgraf et al. 1984, Fay 1991), consuming the plant until it comprises up to 50% of their daily dry matter intake (Landgraf et al. 1984, Bartz et al. 1985). Goats also readily consume the plant (Walker and Kronberg 1992, Sedivec and Maine 1993).

An herbivore may avoid a plant due to plant cell wall characteristics, (including fiber and lignin content), the presence of unpalatable or physiologically adverse

phytochemicals, or the presence of structural defenses (Van Soest 1982, Reichardt et al. 1987, Bryant et al. 1992). Secondary compounds in plants tend to reduce an herbivore's ability to digest nutrients and can be toxic when consumed at certain levels (Feeny 1976). Herbivory elevates defensive chemical concentrations in many plant species (Rhoades 1979, Baldwin 1988, Mihaliak and Lincoln 1989, Khan and Harborne 1990). Because young leaf tissue is generally preferred by herbivores as a high quality food source, plants may increase levels of secondary chemicals in regrowth as an evolutionary response to herbivory. Researchers have also shown that chemical defenses of woody plants vary by plant growth stage and by specific plant parts within growth stages (Reichardt et al. 1984, Clausen et al. 1986, Reichardt et al. 1990).

Juvenile twigs that are produced after severe grazing of woody plants may be more toxic to herbivores than material present prior to browsing (Bryant et al. 1983, Provenza and Malecheck 1984). Herbivores can avoid these higher levels of toxins by browsing selectively. For example, snowshoe hares are able to discriminate between juvenile and adult developmental stages of boreal woody plants based on levels of secondary metabolites present (Bryant and Kuropat 1980). Some forbs also increase levels of secondary defense compounds in regrowth. Spilatro and Mahlberg (1986) determined the concentration of triterpenols in the latex of young and mature *Euphorbia pulcherrima* (poinsettia) leaves. Concentrations of the triterpenol, cycloartenal were about 25% lower in mature and old leaves than in young and expanding leaves. Similarly, alkaloid levels in tall larkspur (*Delphinium occidentale* [S. Watts.] S. Watts.) decline as the plant matures (Ralphs et al. 1988), suggesting that regrowth should contain

higher levels of toxic alkaloids than mature growth.

Leafy spurge latex contains high concentrations of triterpenols (Mahlberg personal comm.) and diterpenols (Evans and Kinghorn 1977, Upadhyay et al. 1978). The diterpene phorbol present in leafy spurge produces intense skin inflammation, eye conjunctivitis, burning of the oral cavity and throat, diarrhea, and gastroenteritis in humans and many animals (Evans and Soper 1978, Schildknecht 1981). Most terpenoids also have detrimental effects on insects, serving as toxins or feeding deterrents (Gershenzon and Croteau 1991). Terpenoids decrease plant palatability to mammalian herbivores and may decrease digestibility as a result of bacteriocidal effects on rumen microorganisms (Nagy and Tengerdy 1968, Gershenzon and Croteau 1991). Other secondary compounds, such as condensed tannins, may be present in leafy spurge. The identity of the specific chemical(s) in leafy spurge that is adversive to herbivorous mammals and insects is not known.

Secondary defense compounds in leafy spurge may have an adverse effect directly on the herbivore (insect or mammal), on rumen microorganisms in ruminants, or both. The objectives of our studies were to determine: (1) if condensed tannin concentrations increase in defoliated leafy spurge, and (2) if material from undefoliated or previously defoliated leafy spurge shoots adversely affects sheep rumen microorganisms and a generalist grasshopper (*Melanoplus sanguinipes*).

CHAPTER 2

EFFECTS OF LEAFY SPURGE ON SHEEP RUMEN MICROORGANISMS

Introduction

Leafy spurge (*Euphorbia esula* L.) is an introduced perennial weed that invades rangelands, displacing native vegetation and thus reducing rangeland carrying capacity (Reilly and Kaufman 1979). Leafy spurge infests more than 1 million hectares in the United States and Canada, primarily in the Northern Great Plains and prairie provinces (Lajeunesse et al. 1995). In Montana, South Dakota and Wyoming, the total annual economic impact of leafy spurge is estimated at $1.95 million; the annual impact in North Dakota is nearly $10 million (Bangsund et al. 1993).

Leafy spurge tissues and latex contain several secondary plant compounds, including terpenoids (Evans and Kinghorn 1977, Mahlberg personal comm.), that inhibit consumption of the plant by many herbivores (Upadhyay 1978, Lym and Kirby 1987). This may explain why cattle generally consume little, if any, leafy spurge when grazing (Lym et al. 1988). However, domestic sheep have the potential to effectively control leafy spurge (Johnson and Peake 1960, Landgraf et al. 1984, Fay 1991), consuming the plant until it comprises up to 50% of their daily dry matter intake (Landgraf et al. 1984, Bartz et al. 1985). Goats also readily consume the plant (Walker and Kronberg 1992, Sedivec and Maine 1993).

In vitro, essential oils of big sagebrush (*Artemisia tridentata*) inhibit growth of

gram-positive and gram-negative microbes taken from the rumens of mule deer (Nagy et al. 1964). Similarly, monoterpene alcohols in Douglas fir (*Pseudotsuga menziesii*) strongly inhibit the rumen microbial activity of sheep and deer, as reflected by a decrease in microbial gas production (Oh et al. 1967). Rumen microorganisms collected from sheep which had not previously consumed Douglas fir needles produced the least gas, presumably because of the monoterpene alcohols.

Secondary defense compounds may also reduce dry matter digestibility. In vivo crude protein digestibility declines significantly when ponderosa pine (*Pinus ponderosa* Laws.) needles are added to crested wheatgrass (*Agropyron desertorum* [Link] Schultes) hay (Adams et al. 1992). This was attributed at least partially to the effects of phenolics or other secondary compounds on rumen microbial populations. Pine needles had a greater effect on rumen microbial digestion in vitro than in vivo.

Similarly, concentrations of extractable polyphenols in 4 Greek browse species are related negatively to gas production and in vivo dry matter disappearance (Khazaal et al. 1992). Increasing concentrations of phenolics had a larger effect on gas production than on dry matter degradation. Therefore, the gas production trial was considered more sensitive than the in vivo nylon bag technique in identifying feeds with antinutritive factors.

Although sheep will consume leafy spurge, they will consume it at levels only up to 50% of their total dry matter intake (Landgraf et al. 1984, Bartz et al. 1985). Our objective was to determine the response of sheep rumen microorganisms to different mixtures of grass hay with undefoliated or defoliated leafy spurge collected 3 times

during the growing season. Our hypothesis was that increasing levels of leafy spurge and defoliated leafy spurge in these mixtures would reduce in vitro dry matter disappearance, microbial gas production, and microbial purine accumulation. Microbial gas production was used as an indicator of microbial activity, whereas microbial purine accumulation was used as an indicator of microbial mass.

Materials and Methods

The effects of leafy spurge on sheep rumen microbial activity and biomass were determined with in vitro dry matter disappearance (IVDMD) and gas production trials at the Oscar Thomas Nutrition Center at Montana State University. Plant material for both trials was collected from a leafy spurge-infested rangeland approximately 17 km southeast of Grass Range, Montana (46° 52' N 108° 52' W), in the foothills of the Big Snowy Mountains. The elevation is about 1,340 m. Annual precipitation averages 400 mm. Mean annual temperature is 6° C. Soils on the site are Castner-Amherst Series loams (loamy-skeletal, mixed Lithic Haploborolls and clayey montmorillonitic Lithic Argiborolls; USDA 1979). These are shallow, well-drained soils derived dominantly from fractured hard sandstone and consolidated shale interbedded with sandstone. The predominant graminoid on the site is Kentucky bluegrass (*Poa pratensis* L.) and dominant forbs include wild rose (*Rosa woodsii* Lindl.), prairie coneflower (*Ratibida columnifera* [Nutt.] Woot. And Standl.), yarrow (*Achillea millefolium* L.), and curlycup gumweed (*Grindelia squarrosa* [Pursh.] Dun.).

We collected samples from a large contiguous area in a heavily infested leafy

spurge stand to minimize variability that soils on the site may have on leafy spurge chemistry. Leafy spurge was defoliated with a weedeater on 15m × 20m plots on May 19, June 23, and July 28 of 1994. Undefoliated leafy spurge shoots were collected on these dates. Undefoliated shoots and regrowth from previously defoliated shoots were harvested by hand 3 weeks later on June 9, July 14, and August 18. Undefoliated shoots were harvested from plots adjacent to the previously defoliated plots on these dates.

Forage Analysis

All leafy spurge material was air dried. Leaves, stems, and flowers, if present, were then separated from undefoliated shoots. Regrowth leaves and stems were separated from previously defoliated shoots. All plant parts were ground to pass through a 1-mm screen in a Wiley mill. Dry matter (DM), ash, crude protein (CP), and ether extract (EE) were determined by standard methods (AOAC 1984). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined by the procedures of Goering and Van Soest (1970). Condensed tannins (CT) were extracted and assayed according to the colorimetric procedure of Burns (1971), as modified by Price et al. (1978).

All leafy spurge plant parts were mixed with low quality grass hay (6.3% CP) in the following proportions: 1) 100:0 (leafy spurge:grass hay), 2) 75:25, 3) 50:50, 4) 25:75, and 5) 0:100 (control). The grass hay was also ground to pass through a 1-mm screen in a Wiley mill. Forage treatments included these 5 mixtures of the separate leafy spurge plant parts collected on each of the 3 sampling dates. Regrowth leaves were not present

on previously defoliated shoots collected in August.

Conventional and Modified In Vitro Trials

Ruminal IVDMD was determined for these forage treatments using the first stage of Tilly and Terry's (1963) conventional in vitro system (n=3 runs). Rumen fluid inoculum for in vitro trials was collected from 3 ruminally fistulated Targhee ewes maintained on a diet of the low quality grass hay previously described. Rumen fluid was composited and strained through 8 layers of cheese cloth. Forage samples were weighed (0.25 g) into 50-ml fermentation tubes. Twenty milliliters of McDougall's buffer (McDougall 1948) was added to each tube, and then each tube was inoculated with 5 ml rumen fluid. Fermentation tubes were flushed with CO_2, capped, and incubated at 39° C. Duplicate fermentation tubes for each forage treatment and blanks were removed from the incubator at 3, 6, 12, and 24 hours postinoculation. Fermentation tubes were then centrifuged at 2,000 rpm for 15 minutes, decanted, and dried at 60° C for 48 hours to determine ruminal IVDMD.

For the same forage treatments, a modified in vitro system was used to measure gas production during fermentation and ruminal IVDMD (Thomas et al. 1994). For each forage treatment, duplicate 250 ml flasks were prepared containing 2 g forage, 100 ml McDougall's buffer, and 50 ml strained rumen fluid. In vitro flasks were then placed in a 39° C shaking water bath and gas production was measured by water displacement in inverted burettes. Gas production readings were taken at 2, 3, 4, 6, 12, and 24 hours. Following the 24-hour reading, contents of the flasks were filtered to separate the residue

from the fluid fraction. Residues were dried at 60° C for 48 hours and weighed to determine IVDMD. Residues were also analyzed for NDF content (Goering and Van Soest 1970) and purine concentration of attached bacteria (Zinn and Owens 1986).

Data Analysis

Changes in DMD and gas production over time, and 24-hour IVDMD in the conventional in vitro trial and the modified in vitro trials, respectively, were analyzed for each collection date using a general linear model (GLM; SAS 1987). To assess changes in IVDMD and gas production over time, we used a multivariate repeated measures analysis of variance with plant part (leaves, flowers, and stems from undefoliated shoots, and leaves and stems from previously defoliated shoots), leafy spurge:grass hay mixture (100:0, 75:25, 50:50, 25:75), and the plant part-mixture interaction as between subject factors, and repeated observations as the within subject factor. To adjust for run effect, data were transformed by dividing the treatment value by the 100% grass hay value for each run. We used planned linear contrasts to compare 24-hour cumulative gas production and IVDMD values from plant parts (Table 1). To compare responses to the different mixtures of leafy spurge and grass hay, linear, quadratic, and cubic regression coefficents were tested, as well as all possible two- and three-way interactions. P-values less than 0.10 are reported (Gill 1981).

For the modified in vitro trial, 24-hour IVDMD, cumulative gas production, and residue NDF fraction and purine concentration were analyzed for each collection date using 100% grass hay responses as covariates. Planned linear contrasts were again used

to compare treatment means for responses to the different plant parts, and linear, quadratic, and cubic contrasts were used to compare responses to the different mixtures of leafy spurge and grass hay. We also determined correlations between CT concentration, DMD, gas production, NDF disappearance (NDFD), and purine concentration (Jandel Scientific 1993).

Table 1. Planned Linear Contrasts (24 hours)

leaves - U vs. stems - U
flowers - U vs. stems - U
leaves - PD vs. stems - PD
leaves - U vs. leaves - PD
stems - U vs. stems - PD

U - plant parts from undefoliated plants
PD - plant parts from previously defoliated plants

Results

Forage Nutrient Composition

The nutritional quality of all plant parts declined as the growing season progressed (Table 2). Regrowth leaves from shoots collected in June and July had higher CP content and lower EE, NDF, ADF and ADL contents than leaves from undefoliated shoots. Regrowth leaves were not present in August. The CP content of grass hay (CP=6.3%) was lower than the CP content of all leafy spurge leaves collected throughout the summer. It was higher than the CP content of leafy spurge stems collected in June, July, and August, and flowers from undefoliated shoots collected in August.

Table 2. Nutritive value of leafy spurge plant parts from plants that were initially defoliated (I) on May 19, June 23, and July 28, and from undefoliated plants (U) and previously defoliated plants (PD) harvested 3 weeks later.

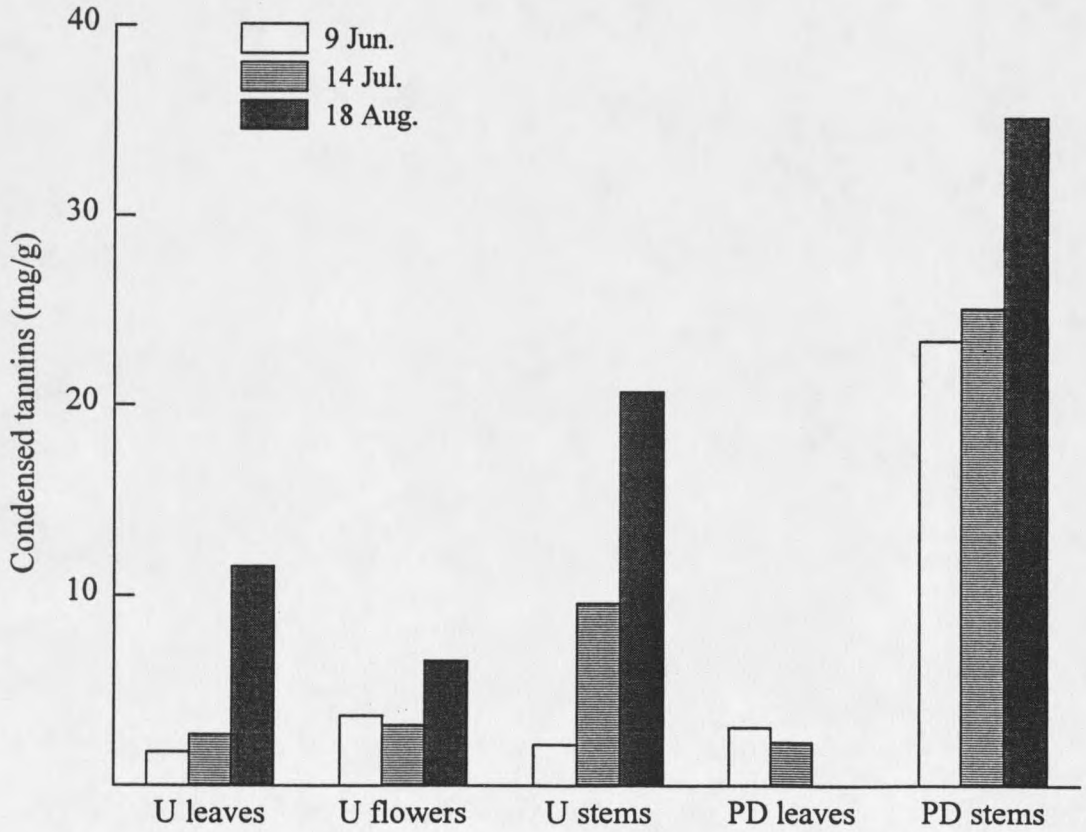
Plant Part	CP	EE	NDF	ADF	ADL
19 May 1994					
I Leaves	22.68	4.18	18.56	15.21	2.94
I Flowers	25.78	5.02	16.98	14.55	3.49
I Stems	9.38	3.03	48.01	40.68	5.96
9 Jun. 1994					
U Leaves	16.59	4.73	18.45	16.87	3.70
U Flowers	17.67	5.28	20.74	18.41	4.73
U Stems	4.85	2.60	63.65	53.64	9.70
PD Leaves	22.22	4.12	16.61	13.31	2.10
PD Stems	6.96	3.32	54.39	47.37	8.51
23 Jun. 1994					
I Leaves	14.52	4.28	26.20	22.91	5.30
I Flowers	14.09	4.98	30.16	25.53	6.56
I Stems	4.22	2.26	65.70	56.39	10.33
14 Jul. 1994					
U Leaves	13.27	4.75	25.89	22.96	5.29
U Flowers	13.23	5.69	31.32	25.59	6.01
U Stems	4.03	2.13	65.44	56.18	10.48
PD Leaves	25.73	3.48	18.69	14.10	2.40
PD Stems	4.08	2.02	71.29	61.99	12.08
28 Jul. 1994					
I Leaves	11.14	5.46	30.69	25.65	6.24
I Flowers	8.65	6.73	36.14	30.45	6.70
I Stems	3.16	2.48	68.17	57.64	11.62
18 Aug. 1994					
U Leaves	8.24	6.55	32.83	28.91	6.36
U Flowers	6.05	6.73	42.60	36.58	7.64
U Stems	2.43	2.57	67.98	60.28	11.53
PD Stems	2.83	2.43	70.38	62.35	12.82

Condensed Tannins

Condensed tannins were present in all leafy spurge plant material collected in

June, July and August (Figure 1). Condensed tannin concentrations of leaves and stems from undefoliated shoots, and stems from previously defoliated shoots were higher on July 14 than on June 9. Condensed tannin concentrations of all leafy spurge plant parts were higher on August 18 than on July 14. Condensed tannin concentrations of stems from previously defoliated shoots were higher than of stems from undefoliated shoots in June, July, and August. Condensed tannin concentrations increased throughout the growing season in all plant parts except in leaves from previously defoliated shoots.

Figure 1. Concentrations of condensed tannins (mg/g) in leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in June, July, and August 1994.



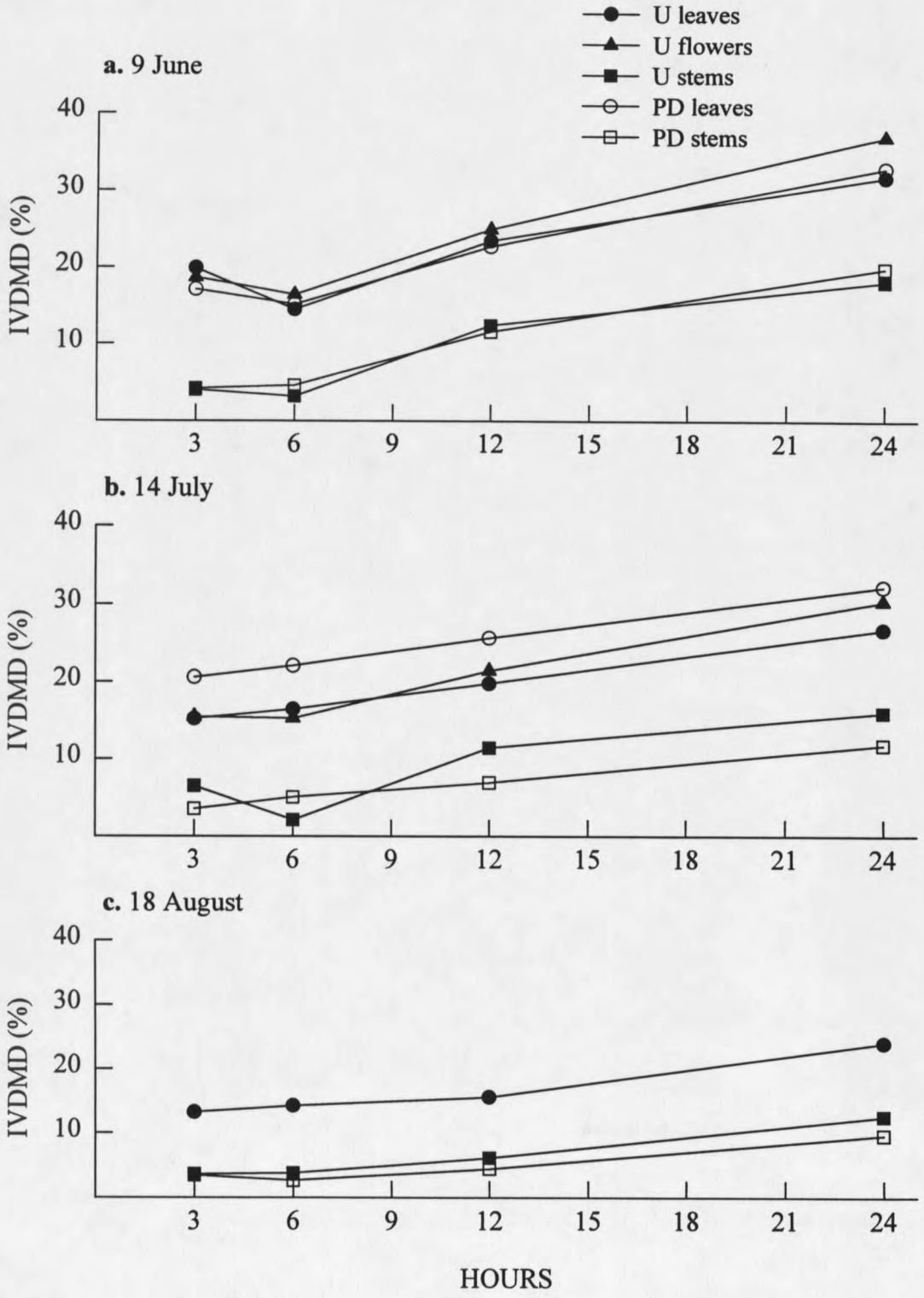
Conventional In Vitro Trial

For plant material collected in June, July, and August, IVDMD varied over the 24-hour period depending on the plant part (time × plant part interaction, $P<0.04$; Figure 2). Over the 24 hour period, IVDMD was higher for leaves and flowers than for stems collected in June, July, and August ($P=0.0001$).

The proportion of leafy spurge in the mixtures affected changes in IVDMD of the plant parts differently over time for material collected in July only (time × plant part × mixture interaction; $P=0.04$). For leaves from undefoliated and previously defoliated shoots, IVDMD increased from 3 to 6 hours for the 50:50 and 25:75 mixtures. However, for flowers from undefoliated shoots, IVDMD decreased from 3 to 6 hours for the 50:50 and 25:75 mixtures. For all leaves and flowers, IVDMD was highest for 100:0 mixtures at all times, and lowest for 25:75 mixtures at all times. For stems from undefoliated shoots, IVDMD of the 75:25 mixtures decreased from 3 to 6 hours; IVDMD increased from 3 to 6 hours for all other mixtures. For stems from previously defoliated shoots, IVDMD increased for all mixtures over time, but was highest for the 25:75 mixture and lowest for the 100:0 mixture.

For undefoliated and previously defoliated shoots collected in June and July, 24-hour IVDMD was higher for leaves and flowers than for stems ($P=0.0001$). For undefoliated shoots collected in August, 24-hour IVDMD was higher for leaves than for stems ($P=0.0001$). For material collected in July, 24-hour IVDMD was higher for leaves from previously defoliated shoots than for leaves from undefoliated shoots ($P=0.0001$). Conversely, stems from undefoliated shoots had higher 24-hour IVDMD than stems from

Figure 2. Dry matter disappearance (%) over a 24-hour period for leafy spurge collected in June, July, and August 1994.



previously defoliated shoots (P=0.0006).

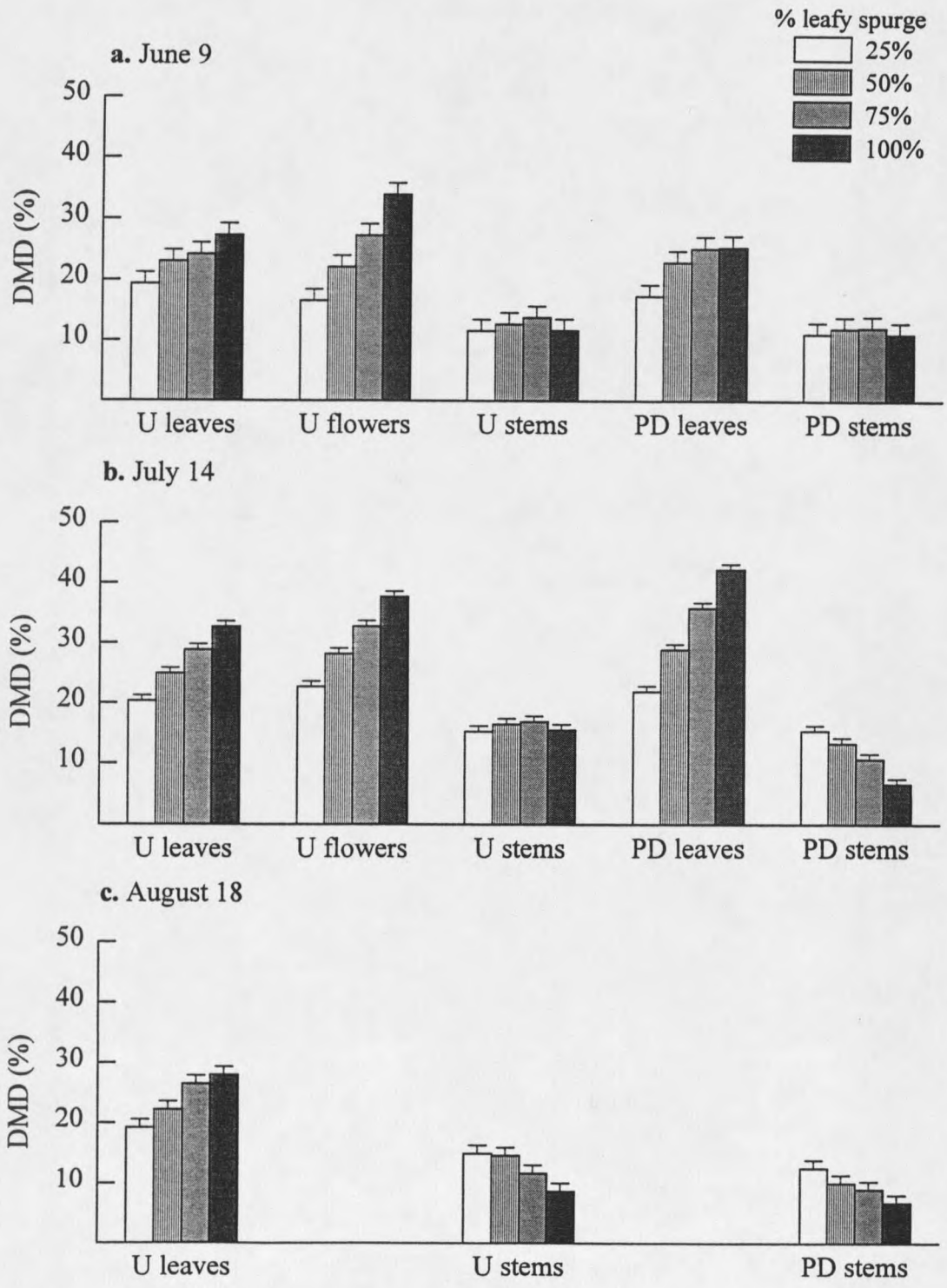
For leaves and flowers from undefoliated and previously defoliated shoots collected in June, July, and August, 24-hour IVDMD increased as proportions of leafy spurge in the mixture increased (linear, P<0.004; Figure 3). In contrast, for stems from defoliated shoots collected in July, 24-hour IVDMD decreased as proportions of leafy spurge in the mixture increased (linear, P=0.0008). Similarly, for stems collected from undefoliated and defoliated shoots in August, 24-hour IVDMD decreased as proportions of leafy spurge increased (linear, P<0.02).

Modified In Vitro Trial

For plant material collected in June and July, microbial gas production varied over the 24-hour fermentation period depending on the plant part and mixture (time × plant part × mixture interaction, P<0.0003). For leaves and flowers from undefoliated and defoliated shoots collected in June, microbial gas production for the 100:0 and 75:25 mixtures was high initially, decreased rapidly, and was then constant between 4 hours and 24 hours. For stems from undefoliated and defoliated shoots collected in June, microbial gas production for the 100:0 and 50:50 mixtures was initially low, increased, and was then constant between 4 and 24 hours. Between 4 and 24 hours, microbial gas production was similar for all proportions.

For leaves and flowers collected in July, microbial gas was produced most rapidly from the 50:50 mixtures. For the 100:0 mixtures of leaves and flowers collected in July, microbial gas was produced rapidly up to 4 hours, but slowed considerably

Figure 3. In vitro dry matter disappearance (%; 24-hour; conventional in vitro system) of 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in: **a.** June, **b.** July, and **c.** August 1994. Least square means ± 1 standard error.



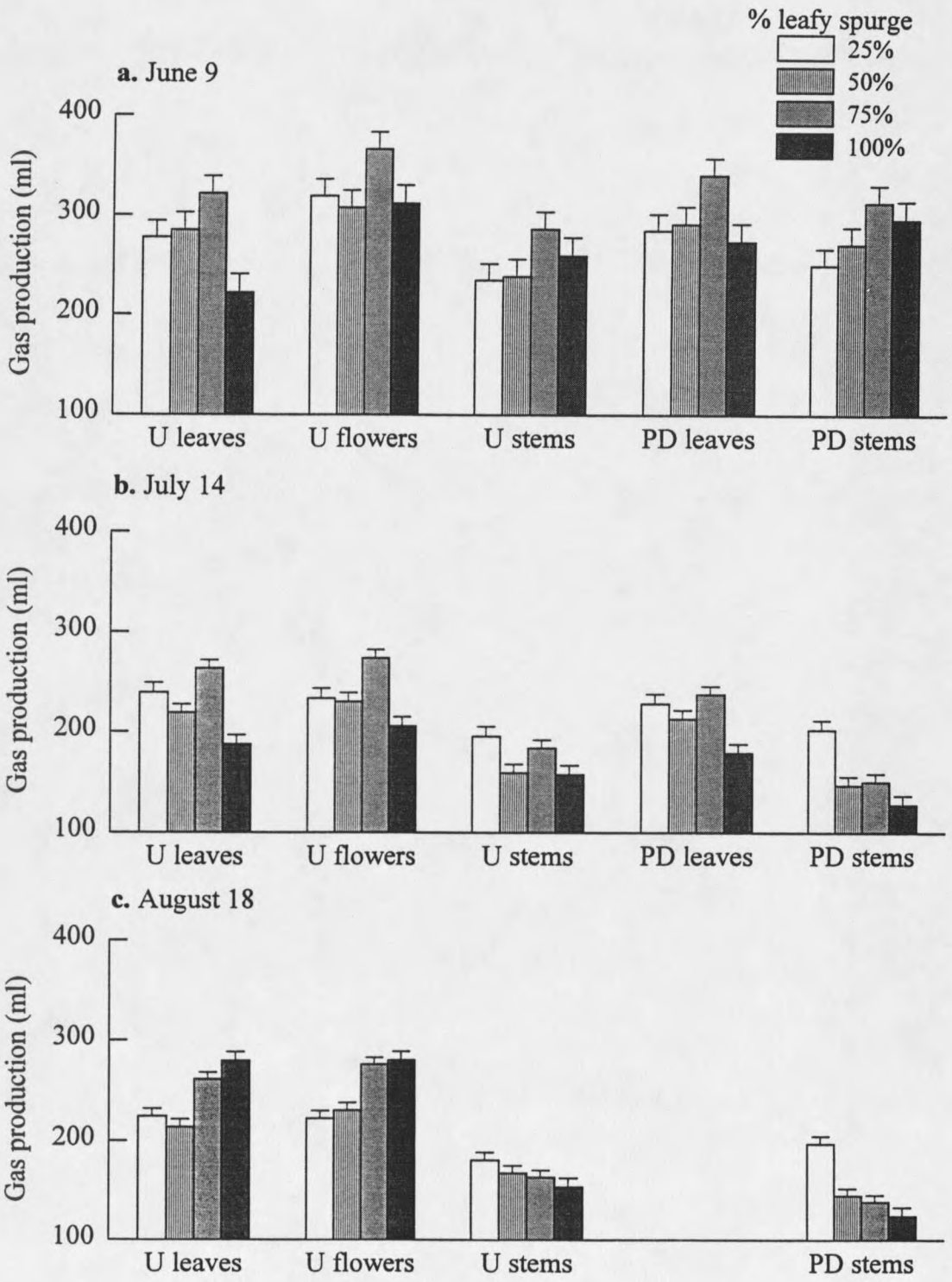
thereafter. For stems, microbial gas production increased between 2 and 4 hours for 100:0 and 25:75 mixtures; gas production for the 50:50 and 75:25 mixtures was relatively constant between 2 and 24 hours.

For all plant parts collected in August, microbial gas production varied over the 24-hour fermentation period depending on the mixture (time × mixture interaction, P=0.0001). Microbial gas was produced slowly for the first 3 hours, increased from 3 to 4 hours, and then remained constant for the 100:0 and 75:25 mixtures. Conversely, for the 50:50 and 25:75 mixtures, rate of microbial gas production increased for the first 3 hours, decreased from 3 to 4 hours, and then remained constant.

For undefoliated and previously defoliated shoots collected in June, July, and August, more microbial gas was produced from leaves and flowers than from stems (planned contrasts; P=0.0001; Figure 4). For material collected in June, more microbial gas was produced by stems from previously defoliated than undefoliated shoots (P=0.0001). Conversely, for material collected in July, more microbial gas was produced by stems and leaves from undefoliated shoots than previously defoliated shoots (P=0.0001, P=0.004, respectively).

For leaves from undefoliated shoots collected in June and July, more microbial gas was produced from the 75:25 mixture than the other mixtures (quadratic, June P=0.05, July P=0.02). For flowers from undefoliated shoots collected in July, more microbial gas was also produced from the 75:25 mixture than the other mixtures (quadratic, P=0.03). For previously defoliated leaves collected in July, the 75:25 mixture

Figure 4. Microbial gas production (ml; 24-hour) of 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in: **a.** June, **b.** July, and **c.** August 1994. Least square means ± 1 standard error.



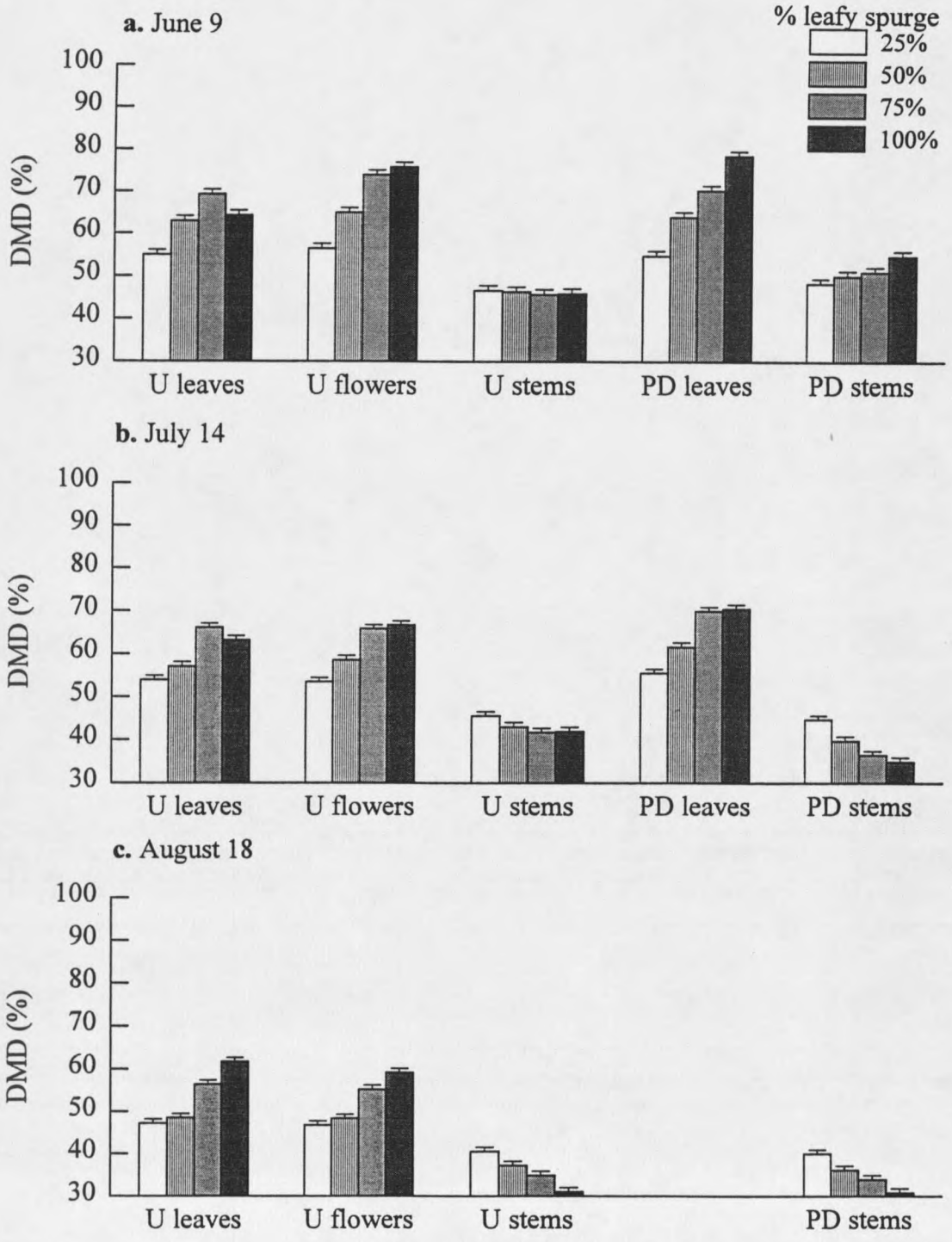
produced the most microbial gas (quadratic, P=0.02). For stems from previously defoliated shoots collected in July, the 25:75 mixture produced the most microbial gas (linear, P=0.003). For leaves and flowers from undefoliated shoots collected in August, microbial gas production increased as the proportion of grass hay increased in the mixture (linear, P<0.002). Conversely, for stems from undefoliated and previously defoliated shoots collected in August, microbial gas production decreased as the proportion of grass hay increased in the mixture (linear, P<0.05).

For undefoliated and previously defoliated shoots collected in June and July, 24-hour DMD was higher for leaves and flowers than for stems (planned contrasts - across mixtures; P=0.0001; Figure 5). For material collected in June, 24-hour DMD was higher for leaves and stems from previously defoliated shoots than for leaves and stems from undefoliated shoots (P=0.0001). Similarly, for material collected in July, 24-hour DMD was higher for leaves from previously defoliated shoots than for leaves from undefoliated shoots (P=0.0001). Conversely, 24-hour DMD was higher for stems from undefoliated shoots than for stems from previously defoliated shoots (P=0.0001).

For leaves from undefoliated shoots collected in June, 24-hour DMD was the highest for the 75:25 mixture (quadratic, P=0.04). For flowers from undefoliated shoots collected in June, increasing the amount of leafy spurge in the mixtures increased 24-hour DMD (linear, P=0.0001). For leaves and stems from previously defoliated shoots collected in June, increasing the amount of leafy spurge in the mixtures also increased 24-hour DMD (linear, P<0.01).

For leaves and flowers from undefoliated and previously defoliated shoots

Figure 5. Dry matter disappearance (%; 24-hour; modified in vitro system) of 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in: **a.** June, **b.** July, and **c.** August 1994. Least square means ± 1 standard error.



collected in July and August, increasing the amount of leafy spurge in the mixtures increased 24-hour DMD (linear, $P<0.02$). In contrast, increasing the amount of leafy spurge stems in the mixtures decreased 24-hour DMD (linear, $P<0.04$).

For each collection date, NDFD was higher for leaves and flowers from undefoliated and previously defoliated shoots than for stems (planned contrasts - across mixtures; $P=0.0001$; Figure 6). For material collected in June, NDFD was higher for leaves and stems from previously defoliated shoots than for leaves and stems from undefoliated shoots ($P=0.0001$). For material collected in July, NDFD was higher for leaves from previously defoliated shoots than for leaves from undefoliated shoots ($P=0.0001$).

For material collected in June, July, and August, more microbial nitrogen was produced from leaves and flowers than stems from undefoliated and previously defoliated shoots ($P<0.0007$; Figure 7). In addition, for material collected in June and July, leaves from previously defoliated shoots produced more microbial nitrogen than leaves from undefoliated shoots ($P=0.0001$). Microbial nitrogen production was similar between stems from undefoliated and previously defoliated shoots collected in June, July, or August.

Microbial gas production, DMD, NDFD, and microbial nitrogen concentrations decreased as CT concentrations increased ($P<0.04$; Table 3). Dry matter disappearance increased as gas production increased ($P=0.003$); however, NDFD and microbial nitrogen concentration were not correlated with gas production ($P=0.2$, $P=0.5$, respectively). Dry matter disappearance increased as NDFD and microbial nitrogen concentration increased

Figure 6. Neutral detergent fiber disappearance (%; 24-hour; modified in vitro system) of 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in: **a.** June, **b.** July, and **c.** August 1994. Least square means ± 1 standard error.

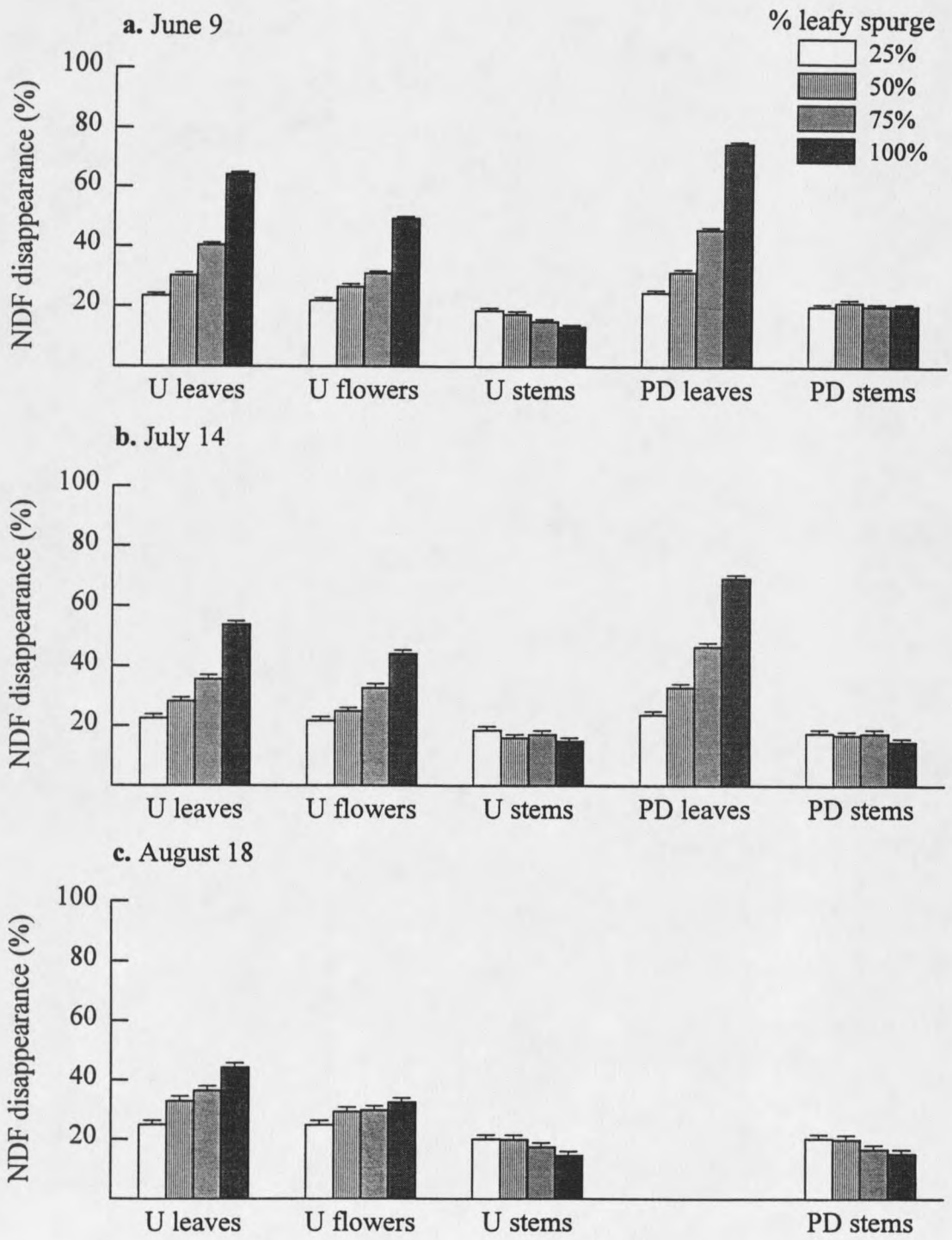
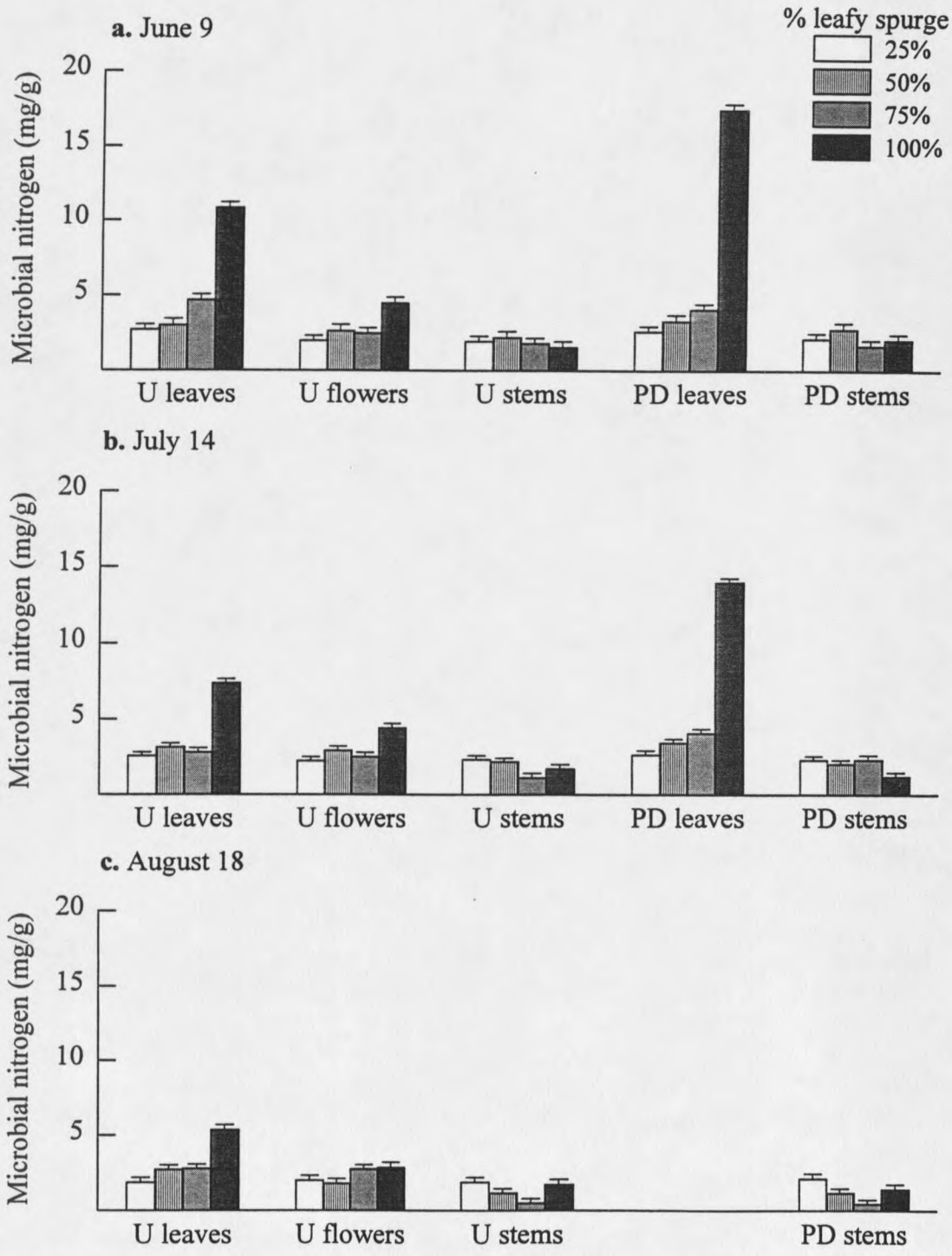


Figure 7. Microbial nitrogen (mg/g) of 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in **a.** June, **b.** July, and **c.** August 1994. Least square means ± 1 standard error.



($P<0.0001$, $P=0.003$, respectively). Similarly, NDFD increased as microbial nitrogen concentration increased ($P<0.0001$).

Table 3. Correlation coefficients (r) of the relationships between CT concentrations, microbial gas production, DMD, NDFD, and microbial N concentrations. n = 14.

	Correlation coefficient with			
	CT	*Gas production*	*DMD*	*NDFD*
Microbial gas production	-0.60			
DMD - 24 hour	-0.76	0.73		
NDFD	-0.67	0.35	0.88	
Microbial N concentration	-0.55	0.19	0.73	0.92

Discussion

Condensed Tannins

Condensed tannins were present in leaves, flowers, and stems of leafy spurge. Condensed tannins suppress voluntary forage intake of ruminants by inhibiting microbial growth and activity in the rumen (Smart et al. 1961, Barry and Duncan 1986) and mammalian enzymes in the small intestines (Kumar and Singh 1984, Butler et al. 1986, Clausen et al. 1989). High concentrations of CT in grasslands 'Maku' lotus (*Lotus pedunculatus* L.) depress rumen carbohydrate digestion and voluntary intake in sheep (Barry and Duncan 1984). Similarly, condensed tannins inhibit cellulose digestion by the ruminal bacteria *Fibrobacter succinogenes* (Bae et al. 1993).

Rumen fiber digestion in sheep is depressed when CT concentration in the diet is

about 4% DM, whereas intake and growth are depressed when CT concentration is 6-10% DM (Barry and Duncan 1984, Barry 1985, Barry et al. 1986). Voluntary intake by kudus, impalas, and goats decreases when leaves of South African plant species contain CT concentrations above 5% DM (Cooper and Owen-Smith 1985). Condensed tannin concentrations in leafy spurge were frequently at or above these concentrations, particularly in August. They exceeded 20% DM in previously defoliated stems in June, July, and August. Sheep seldom consume leafy spurge stems, especially in mid- and late summer (Personal observation). Condensed tannins in leafy spurge may inhibit voluntary intake and fiber digestion at certain levels in domestic sheep. In our trials, condensed tannin concentrations were negatively correlated with microbial gas production, DMD, NDFD, and microbial nitrogen concentrations.

The presence of CT in leafy spurge may also deter herbivory by cattle. Generalist grazers, including domestic cattle, generally encounter low levels of secondary compounds in their diet and have not evolved mechanisms to metabolize these compounds. Conversely, specialist grazers and browsers typically consume forages containing high concentrations of secondary compounds. Sheep, goats and mule deer produce high-proline tannin-binding proteins in their saliva that allow them to utilize tanniferous forages in varying quantities (Robbins et al. 1987, Austin et al. 1989, Mehanso et al. 1992). Cattle do not produce these salivary proteins (Jones and Mangan 1977, Austin et al. 1989).

Concentrations of CT increased in leafy spurge stems after the plant was defoliated. Defoliation-induced responses have been observed in other forage species.

Condensed tannin concentrations increase in blackbrush (*Coleogyne ramosissima*) after browsing by goats (Provenza and Malecheck 1984). Long-term defoliation by budworms increases concentrations of CT in current-year needles of Douglas fir trees (Walters and Stafford 1984). Conversely, clipping or browsing does not induce the production of CT in several Alaskan tree and shrub species and Mediterranean woodland species (Chapin et al. 1985, Perevolotsky 1994). Condensed tannin concentrations may have increased in leafy spurge stems after defoliation but not in leaves because more carbon is available in stem tissue for producing CT; CT are immobile within the plant (Skogsmyr and Fagerström 1992).

In June, CT concentrations were highest in flowers of leafy spurge. However, in July and August, concentrations were highest in stems. Other researchers have also noted differences in CT concentrations between specific plant parts. Flower tissues of sulla (*Hedysarum coronarium*) sampled in late spring contain much higher concentrations of CT than leaf or stem tissues (Terrill et al. 1992). Flowers may contain higher concentrations of CT early in the growing season to defend developing reproductive tissues. This may explain why leafy spurge flowers contained higher concentrations of CT than leaves and stems in June.

Condensed tannin concentrations also increased seasonally in all leafy spurge plant parts. Condensed tannin concentrations in sericea (*Sericea lespedeza*) increase as the plant matures (Burns 1966, Cope et al. 1971); they are low when active growth begins, increase until mid-July, and then decrease to initial concentrations in mid-October (Cope et al. 1971). Condensed tannins also increase seasonally in birdsfoot trefoil (*Lotus*

corniculatus) and big trefoil (*Lotus uliginosus*) (Chiquette et al. 1989, Lees et al. 1994). Early in the growing season, leafy spurge may allocate more nutrients to growth and reproduction than to the production of tannins, which is associated with very high metabolic costs (Skogsmyr and Fagerström 1992). Condensed tannins are carbon-based compounds; increases in the C/N ratio later in the growing season may provide more carbon for producing CT in leafy spurge.

Microbial Activity

We frequently observed associative effects by adding leafy spurge to grass hay. Associative effects are nonlinear responses in nutrient utilization that occur as a result of combining 2 feedstuffs in ruminant diets (Moe 1979, Hassan et al. 1988). Positive associative effects may occur because of increased microbial activity, whereas negative associative effects may occur because of reduced rates of fiber digestion (Hart 1987).

In June and July, we observed positive associative effects in microbial gas production by adding leafy spurge leaves, flowers, and stems to grass hay. Microbial gas production was generally the highest for the 75:25 mixtures. Particularly early in the growing season, these results indicate that adding leafy spurge to grass hay stimulates rumen microbial activity in sheep until 75% of their total diet is leafy spurge. Generally, 100% leafy spurge diets were associated with the lowest microbial activity compared with other mixtures. Microbial activity may be inhibited at 100% leafy spurge levels because only those microbes already adapted to compounds in leafy spurge are active. Sheep rumen microorganisms used in these trials were not previously exposed to leafy spurge.

A change in diet is probably the most important factor influencing numbers and relative proportions of different microbe species in the rumen (Yokoyama and Johnson 1988). This involves an adaptation period of several days to several weeks. In the presence of some grass hay, microbial populations that are not adapted to leafy spurge may preferentially utilize that substrate initially, therefore explaining the higher microbial activity observed for mixtures containing 75% grass hay.

In August, microbial gas production increased with increasing quantities of leafy spurge leaves and flowers added to grass hay, presumably because of the relatively higher CP content of leaves and flowers of leafy spurge compared with grass hay. Conversely, microbial gas production decreased with increasing levels of stems from leafy spurge added to grass hay; the higher CP content of grass hay relative to leafy spurge stems, or the high CT concentrations in stems may have reduced microbial gas production.

For all plant parts collected in August, microbial gas production varied over the 24-hour fermentation period depending on the leafy spurge mixture, presumably because of differences in CP and fiber content, and high CT concentrations in leafy spurge, which may have slowed microbial activity in response to the substrate. The delayed or reduced microbial response may reflect a rapid change in species composition (Yokohama and Johnson 1988) when first exposed to high levels of leafy spurge. This would account for slow microbial gas production in early hours of fermentation.

Microbial mass

Microbial mass, as indicated by purine concentration, increased as the proportion

of leafy spurge leaves and flowers increased in the mixtures. Thomas et al. (1994) detected no differences in ruminal purine concentrations between sheep fed 100% grass hay and 50% leafy spurge / 50% grass hay diets. In our study, differences in microbial mass were minimal between 50:50 and 25:75 treatments. However, microbial mass for 100:0 leafy spurge leaves and flowers was considerably greater than microbial mass produced by the other mixtures.

Microbial species that are tolerant of leafy spurge may increase rapidly when leafy spurge is added to the diet, especially when species that are sensitive to leafy spurge are eliminated. This may account for large increases in microbial mass at the 100% leafy spurge levels. On the other hand, dead microorganisms may accumulate when high levels of leafy spurge are in the diet, which would inflate values for those treatments.

Disappearance

Trends in IVDMD were similar for the conventional and modified in vitro systems. However, for the modified in vitro system, 24-hour IVDMD values for all plant parts and mixtures were approximately 2 times higher than those for the conventional in vitro system. We innoculated flasks in the modified system with twice as much rumen fluid as tubes in the conventional system so that gas production could be recorded. In addition, residues from the modified system were rinsed, accounting for slightly higher IVDMD values than those from the conventional system.

Adding leafy spurge leaves and flowers to grass hay increased IVDMD, whereas adding leafy spurge stems to grass hay decreased IVDMD. The higher CP and lower

NDF content of leaves and flowers from leafy spurge compared with grass hay may account for the increases in IVDMD, whereas the lower CP and higher NDF content of leafy spurge stems compared with grass hay may account for the decreases in IVDMD. Similar results have been reported for other high-protein supplements and forage species, including tyfon (*Brassica campestris* var. *rapa* L. × B. *pekinensis* [Lour.] Rupr.) (Cassida et al. 1994) and corn (Chase and Hibberd 1987) when compared with low quality hay. However, this contrasts with the negative associative effect for DMD of rape (*Brassica napus*) (Lambert et al. 1987).

Negative associative effects on fiber digestibility have been reported for lambs fed rape and hay (Lambert et al. 1987) and cattle fed turnips and straw (MacDearmid et al. 1983, Williams et al. 1983). However, adding leafy spurge leaves and flowers to grass hay increased NDFD, indicating that cellulolytic bacteria were not adversely affected by compounds in leafy spurge. Conversely, adding leafy spurge stems to grass hay tended to decrease NDFD; stems of leafy spurge generally had slightly higher NDF content than grass hay.

Conclusions

In June, July, and August, increasing levels of leafy spurge leaves and flowers increased IVDMD, NDFD, and microbial mass, and increased microbial activity up to the 75% leafy spurge level. Therefore, relatively high levels of leafy spurge leaves and flowers did not adversely affect sheep rumen microorganisms. Early in the growing season, IVDMD, NDFD, microbial activity and microbial mass were higher for leaves

from previously defoliated shoots than for leaves from undefoliated shoots. Therefore, it also appears that leaves from previously defoliated leafy spurge plants do not have a negative impact on sheep rumen microorganisms.

In July and August, increasing levels of leafy spurge stems decreased IVDMD and microbial activity, and tended to decrease NDFD and microbial mass. In addition, IVDMD and microbial activity were lower for stems from previously defoliated shoots than from undefoliated shoots in July. These responses correlate with considerable increases in CT concentrations in stems, possibly explaining why sheep avoid leafy spurge stems in their diets, especially late in the growing season.

Concentrations of CT in leafy spurge increased seasonally and were higher in stems from previously defoliated shoots than in stems from undefoliated shoots. For this study only 1 site was sampled; thus, these effects cannot be inferred to all leafy spurge populations. However, we observed similar trends in CT concentrations following defoliation on another field site (Chapter 3). In addition, we did not isolate secondary compounds, such as the specific terpenoids or CT in leafy spurge to determine their individual effects on sheep rumen microorganisms. Therefore, synergistic effects of condensed tannins and other chemical defense compounds in leafy spurge, including terpenoids, may affect the overall response of sheep rumen microorganisms to the plant.

CHAPTER 3

EFFECTS OF DEFOLIATING LEAFY SPURGE ON MIGRATORY GRASSHOPPER NYMPHS

Introduction

Leafy spurge (*Euphorbia esula* L.) is an introduced perennial weed that infests millions of hectares in the Northern Great Plains (Lacey et al. 1985). Biological control of leafy spurge has had limited success because compounds in the plant latex inhibit consumption by many herbivores (Lym and Kirby 1987). Secondary compounds in plants tend to reduce an herbivore's ability to digest nutrients and can be toxic when consumed at certain levels (Feeny 1976). In addition, herbivory increases the production of secondary compounds in many plant species (Rhoades 1979, Harborne 1986). However, the effects of herbivory on the concentrations of secondary compounds in leafy spurge have not been documented.

Leafy spurge latex contains large concentrations of terpenoids (Mahlberg personal comm., Evans and Kinghorn 1977), most of which have detrimental effects on insects as toxins or feeding deterrents (Gershenzon and Croteau 1991). Condensed tannins (CT) are also present in the plant (Chapter 2). These polyphenols have the ability to bind proteins and most likely deter herbivory with their astringent taste (Harborne 1986). Furthermore, CT may have a structural defense role, contributing to leaf toughness and woodiness (Haslam 1988).

The effects of secondary plant compounds on several grasshopper species have been reported. Effects of 20 secondary plant chemicals on survival, development, and feeding behavior of the forbivorous grasshopper, *Melanoplus bivittatus* (Say) were determined; the triterpenoid saponin was lethal (Harley and Thorsteinson 1967). Similarly, diets containing more than 10% saponin inhibit feeding of *Locusta migratoria* by 50% (Bernays and Chapman 1977). As concentrations of many compounds were increased incrementally, feeding decreased.

Neonate grasshoppers (nymphs) are more susceptible to diet than adults (Westcott et al. 1992). Survival and mean weight of *M. sanguinipes* nymphs varied when feeding on several different cereals (Hinks et al. 1987). Nymphs of *M. sanguinipes* were exposed to 22 secondary plant compounds; alkaloids, flavonoids, phenolics, and terpenoids decreased mean weights after a 5-day bioassay (Westcott et al. 1992). Only phenolics had no effect on nymph survival.

Concentrations of secondary compounds may increase after defoliation, therefore deterring subsequent herbivory (Rhoades 1979, Baldwin 1988, Mihaliak and Lincoln 1989, Khan and Harborne 1990). Graminivorous grasshopper herbivory increases phenolic concentrations in western wheatgrass (*Pascopyrum smithii*) by 43% (Redak and Capinera 1994). Grasshoppers may readily consume old leaves of kochia (*Kochia scoparia* L.), but avoid new growth due to increased concentrations of alkaloids or other antifeedants in the new growth (Hinks et al. 1991, Olfert et al. 1990).

Melanoplus bivittatus, a species that prefers forbs, consume leafy spurge late in the growing season when other forages are limited or have low nutritional value (Personal

observation). Objectives of this study were to determine: 1) if levels of CT increase in leafy spurge following defoliation, and 2) the response of nymphs of the generalist feeder *M. sanguinipes* to different mixtures of grass hay with undefoliated or previously defoliated leafy spurge collected 2 times during the growing season.

Materials and Methods

Condensed tannin concentrations were determined at the Oscar Thomas Nutrition Center at Montana State University. The effects of leafy spurge on mortality and mean weight of surviving grasshopper nymphs were determined in bioassays at the Plant Growth Center. A nondiapause strain of *M. sanguinipes* was used in all bioassays. Grasshopper nymphs were hatched at South Dakota State University, sent to Bozeman within 24 hours, and used in our trials when they were 2-3 days old. Nymph weights at the start of all bioassays averaged 0.36 ± 0.1 mg (SE).

Plant material for the trials was collected from a leafy spurge-infested site in the Story Hills, east of Bozeman, Montana (45° 41' N 111° 00' W.). The elevation is 1,525 m. Annual precipitation averages 500 mm. Mean annual temperature is 5°C. Soils on the site are loams and clay loams (fine, mixed Argic Cryoborolls; MSU and USDA SCS 1982). These are deep, well-drained soils derived primarily from alluvium and glacial till of various rock types. The predominant graminoids on the site are Kentucky bluegrass (*Poa pratensis* L.), western wheatgrass (*Agropyron smithii* Rydb.) and Idaho fescue (*Festuca idahoensis* Elmer), and dominant forbs include wild rose *(Rosa woodsii* Lindl.) and tall larkspur (*Delphinium occidentale* [S. Wats.] S. Wats.). The site is also

dominated by Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), snowberry (*Symphoricarpos albus* [L.] Blake) and ponderosa pine (*Pinus ponderosa* [Dougl.] Lawson).

We collected samples from a large contiguous area in a heavily infested leafy spurge stand to minimize variability that soils on the site may have on chemistry of leafy spurge. Leafy spurge was defoliated with a weedeater on 15m × 15m plots on June 22 and July 27, 1995. Undefoliated leafy spurge shoots were collected on these dates. Undefoliated shoots and regrowth from previously defoliated shoots were harvested by hand 4 weeks later, on July 20 and August 24. Undefoliated shoots were harvested from plots adjacent to the previously defoliated plots on these dates.

Forage Analysis

All leafy spurge material was air dried. Leaves, stems, and flowers, if present, were then separated from undefoliated shoots. Regrowth leaves and stems were separated from previously defoliated shoots. All plant parts were ground to pass through a 1-mm screen in a Wiley mill. Dry matter (DM), ash, crude protein (CP), and ether extract (EE) were determined by standard methods (AOAC 1984). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined by the procedures of Goering and Van Soest (1970). Condensed tannins were extracted and assayed according to the colorimetric procedure of Burns (1971), as modified by Price et al. (1978).

All leafy spurge plant parts were mixed with grass hay (6.72% CP) in the following proportions: (1) 100:0 (leafy spurge:grass hay), (2) 75:25, (3) 50:50 (4) 25:75, and (5) 0:100 (control). The grass hay was also ground to pass through a 1-mm screen in a Wiley mill. Forage treatments included these 5 mixtures of the separate leafy spurge plant parts collected on each of the 2 sampling dates and the grass hay control.

Grasshopper Bioassays

Pre-trial nymphal weights were determined by averaging the weights of 6 randomly selected groups of 20 nymphs. Twenty 2-3-day-old grasshopper nymphs were placed in acetate tubes 27 cm in length and 7.15 cm in diameter. Ends of the tubes were closed with rings holding aluminum mesh screen over the opening. Forage treatments (5 g) were weighed into each tube to provide an ad libitum diet for the nymphs for the duration of the trial. The tubes were placed in an incubator maintained at 28 ± 2°C under constant light. Water was provided by placing a small piece of iceberg lettuce core into each tube daily. After 5 days, surviving grasshoppers were frozen for 6 hours. Frozen grasshoppers were warmed to room temperature, counted, and weighed as groups. Average nymph weights for each group were determined. Treatments were replicated 6 times.

Data Analysis

Mean nymph weight and mortality were analyzed for each collection date with the main effects of plant part, mixture, and their interactions with analysis of variance (ANOVA) using general linear models (GLM; SAS 1987). Pre-trial nymph weights, and

nymph responses to the 100% grass hay diet were used as covariates. We used planned linear contrasts to compare responses to plant parts (Table 4). Linear, quadratic, and cubic regression coefficients were determined to compare responses to the different mixtures of leafy spurge and grass hay. P-values less than 0.15 are reported (Gill 1981). We also determined correlations between CT concentration, and mean weight and mortality of the nymphs.

Table 4. Planned Linear Contrasts

leaves - U vs. stems - U
flowers - U vs. stems - U
leaves - PD vs. stems - PD
leaves - U vs. leaves - PD
stems - U vs. stems - PD
leaves and stems - U vs. leaves and stems - PD

U - plant parts from undefoliated plants
PD - plant parts from previously defoliated plants

Results

Forage Nutrient Composition

The nutritive value of all plant parts declined as the growing season progressed (Table 5). Leaves from previously defoliated shoots collected in July and August had higher CP content and lower EE, NDF, ADF, and ADL contents than leaves from undefoliated shoots. The CP content of the grass hay (6.72%) was lower than the CP content of leafy spurge leaves and flowers collected June through August. It was higher than the CP content of leafy spurge stems collected during this period.

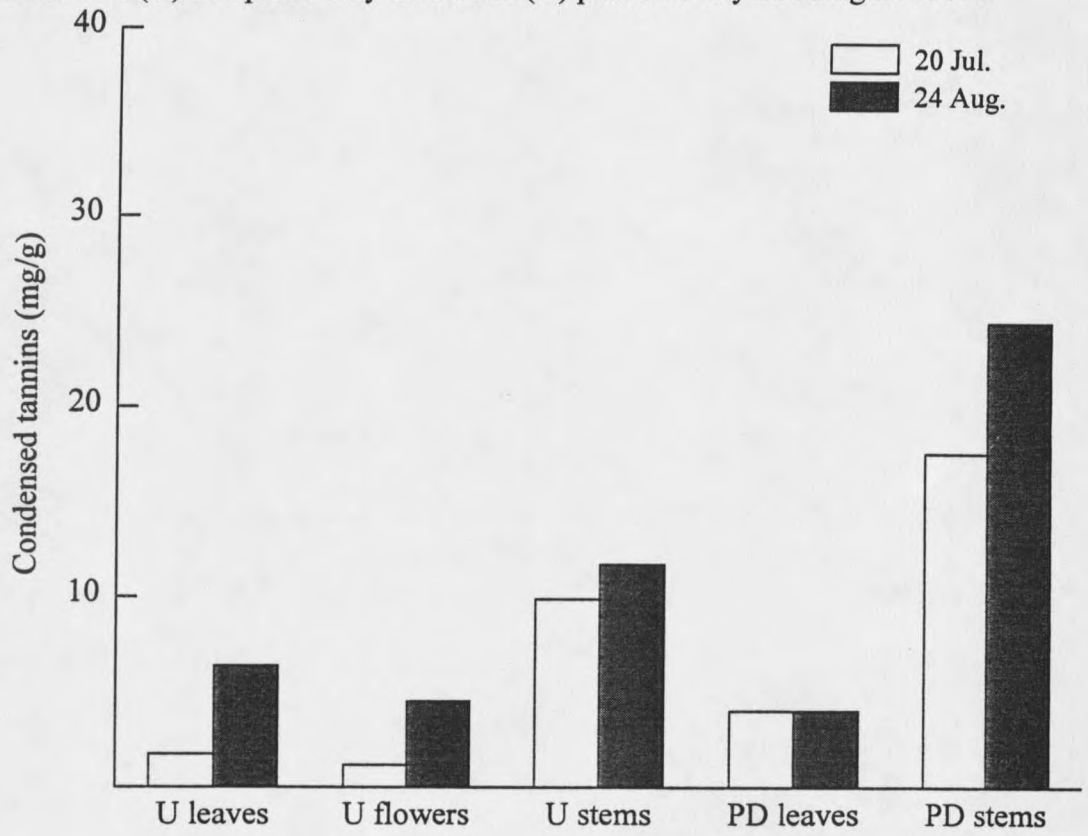
Table 5. Nutritive value of leafy spurge plant parts from plants that were initially defoliated (I) on June 22 and July 27, and from undefoliated plants (U) and previously defoliated plants (PD) harvested 4 weeks later.

Plant Part	CP	EE	NDF	ADF	ADL
22 Jun. 1995					
I Leaves	17.98	4.40	20.86	18.07	4.46
I Flowers	19.34	4.59	22.80	19.13	4.59
I Stems	7.03	2.54	57.32	48.29	9.17
20 Jul. 1995					
U Leaves	11.87	5.24	22.91	20.34	5.08
U Flowers	12.71	5.62	33.27	27.87	7.83
U Stems	4.42	2.53	61.98	54.05	10.43
PD Leaves	16.28	4.94	20.01	17.07	3.46
PD Stems	4.62	2.60	62.10	53.64	10.94
27 Jul. 1995					
I Leaves	11.30	5.09	27.36	24.20	5.75
I Flowers	11.01	5.72	34.34	30.13	7.91
I Stems	4.14	2.60	59.25	53.11	10.21
24 Aug. 1995					
U Leaves	8.79	6.15	29.51	24.63	6.00
U Flowers	8.20	6.45	32.70	28.02	6.52
U Stems	2.68	2.22	65.73	57.62	11.57
PD Leaves	19.39	4.53	19.45	15.73	3.23
PD Stems	2.90	2.84	66.17	56.34	12.10

Condensed Tannins

Condensed tannins were present in all leafy spurge material collected in July and August (Figure 8). Condensed tannin concentrations were higher in August than in July in all plant parts except previously defoliated leaves. Condensed tannin concentrations of previously defoliated stems were higher than those of undefoliated stems in July and August.

Figure 8. Concentrations of condensed tannins (mg/g) in leafy spurge plant parts from undefoliated (U) and previously defoliated (D) plants in July and August 1995.

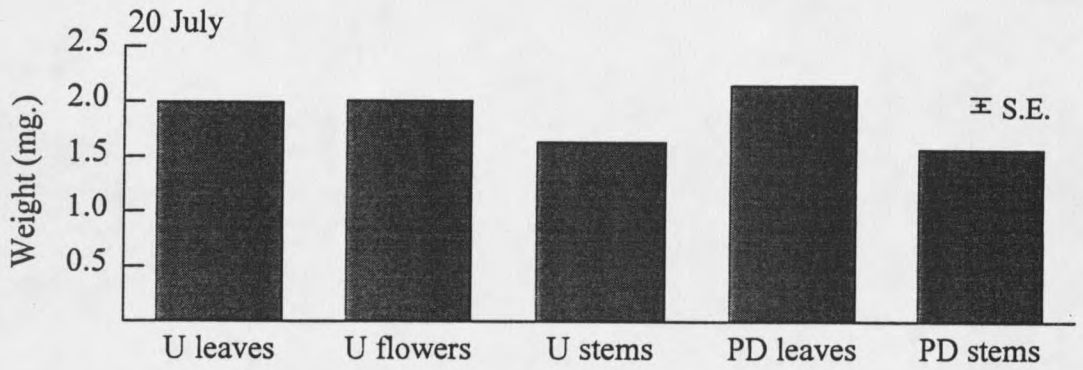


Grasshopper Bioassays

For plant material collected in July, mean grasshopper nymph weight varied depending on the plant part (P=0.0001; Figure 9). Nymphs that consumed leaves from previously defoliated shoots weighed more than nymphs that consumed leaves from undefoliated shoots (P=0.04). Nymphs that consumed leaves and flowers from undefoliated shoots weighed more than nymphs that consumed stems from undefoliated shoots (P=0.0001, P=0.0001, respectively). Nymphs that consumed leaves from previously defoliated shoots weighed more than nymphs that consumed stems from

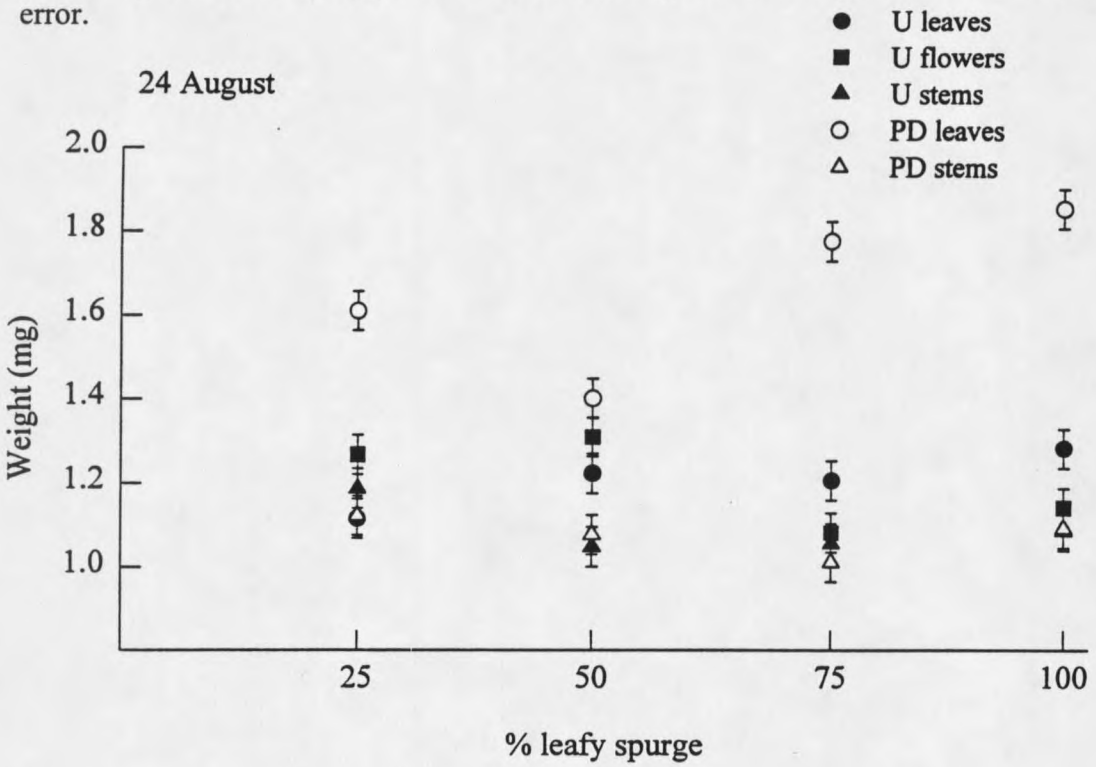
previously defoliated shoots (P=0.0001). Nymphs that consumed leaves and stems from previously defoliated shoots weighed more than nymphs that consumed leaves and stems from undefoliated shoots (P=0.0001).

Figure 9. Mean weights (mg; end of trial) of grasshopper nymphs that consumed leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in July. Least square means ± 1 standard error.



For plant material collected in August, mean grasshopper nymph weight varied depending on plant part and proportion of leafy spurge in the mixture (plant part × mixture interaction; P=0.0001; Figure 10). For nymphs that consumed leaves from undefoliated shoots, mean weights increased as the proportion of leafy spurge in the mixture increased (P=0.08). Conversely, for nymphs that consumed flowers from undefoliated shoots, mean weights decreased as the proportion of leafy spurge in the mixture increased (P=0.0003). For leaves from previously defoliated shoots, mean weights were highest for nymphs that consumed the 100:0 mixture and lowest for nymphs that consumed the 50:50 mixture (quadratic, P=0.04).

Figure 10. Mean weights (mg; end of trial) of grasshopper nymphs that consumed 4 mixtures (leafy spurge:grass hay) of leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in August. Least square means ± 1 standard error.



Mean weights were the highest for nymphs that consumed the 25:75 mixture of stems from undefoliated shoots and lowest for those that consumed the 50:50 mixture (quadratic, P=0.05). Mean weights were again the highest for nymphs that consumed the 25:75 mixture of stems from previously defoliated shoots, but were the lowest for nymphs that consumed the 75:25 mixture (quadratic, P=0.05).

Nymphs that consumed leaves and flowers from undefoliated shoots weighed more than nymphs that consumed stems from undefoliated shoots (P=0.002, P=0.001, respectively). Nymphs that consumed leaves from previously defoliated shoots weighed more than nymphs that consumed stems from previously defoliated shoots (P=0.0001).

Nymphs that consumed leaves from previously defoliated shoots weighed more than nymphs that consumed leaves from undefoliated shoots (P=0.0001). Nymphs that consumed leaves from undefoliated and previously defoliated shoots weighed more than nymphs that consumed stems from undefoliated and previously defoliated shoots (P=0.0001).

For plant material collected in July, grasshopper nymph mortality tended to differ among plant parts (P=0.12; Figure 11), with higher mortality for nymphs with stems in their diet. For plant material collected in August, effect of plant part on mortality was stronger (P=0.004). Nymphs that consumed leaves and flowers from undefoliated shoots had lower mortality than nymphs that consumed stems from undefoliated shoots (P=0.01, P=0.001, respectively). Nymphs that consumed stems from undefoliated shoots had higher mortality than nymphs that consumed stems from previously defoliated shoots (P=0.08). Nymphs that consumed leaves from undefoliated and previously defoliated shoots had lower mortality than nymphs that consumed stems from undefoliated and previously defoliated shoots (P=0.07). Percentage of leafy spurge in the mixture did not affect nymph mortality (July P=0.57, August P=0.39).

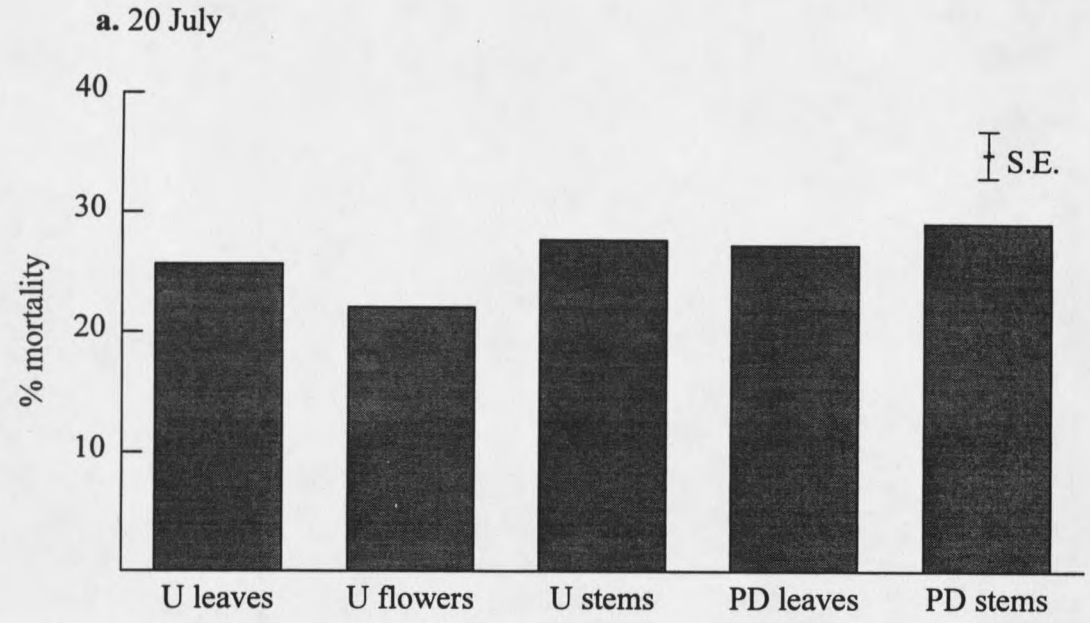
Nymph weight decreased as CT concentrations increased (P=0.06; Table 6). Condensed tannin concentrations and nymph mortality were not correlated (P>0.5).

Discussion

Condensed Tannins

In July, concentrations of CT increased in leafy spurge leaves and stems after the

Figure 11. Percent mortality (end of trial) for grasshopper nymphs that consumed leafy spurge plant parts from undefoliated (U) and previously defoliated (PD) plants harvested in: **a.** July, and **b.** August 1995. Least square means ± 1 standard error.



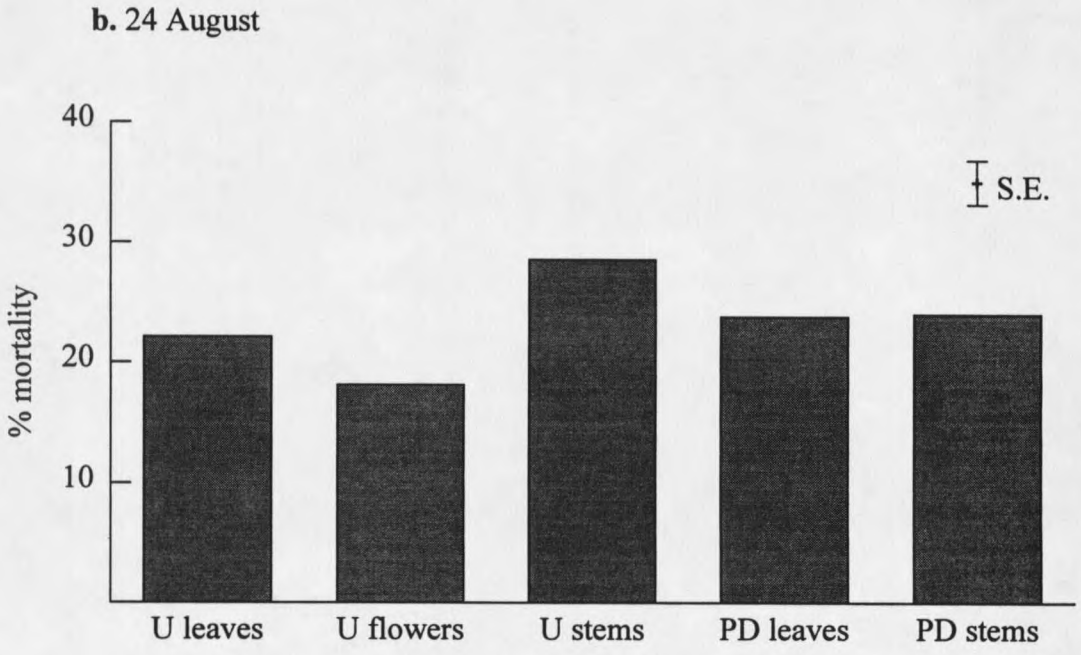


Table 6. Correlation coefficients (r) of the relationships between CT concentrations, nymph weights (mg), and nymph mortality (%). n = 10.

	Correlation coefficient with
	CT concentration
Weight	-0.60
Mortality	0.20

plant was defoliated; in August, concentrations increased only in stems after the plant was defoliated. Defoliation-induced responses have been observed in other plant species. Condensed tannin concentrations increase in blackbrush (*Coleogyne ramosissima*) after browsing by goats (Provenza and Malecheck 1984). Long-term defoliation by budworms increases concentrations of CT in current-year needles of Douglas fir trees (Walters and Stafford 1984). Conversely, clipping or browsing does not induce the production of CT in several Alaskan tree and shrub species, and Mediterranean woodland species (Chapin et al. 1985, Perevolotsky 1994). In August, CT concentrations may have increased in leafy spurge stems after defoliation but not in leaves because more carbon is available in stem tissue for producing CT; CT are immobile within the plant (Skogsmyr and Fagerström 1992).

In July and August, CT concentrations were higher in stems than leaves. Other researchers have also noted differences in CT concentrations between specific plant parts. Flower tissues of sulla (*Hedysarum coronarium*) sampled in late spring contain much higher concentrations of CT than leaf or stem tissues (Terrill et al. 1992). Flowers may contain higher concentrations of CT early in the growing season to defend developing

reproductive tissues. We did not collect leafy spurge early in the growing season. However, in a previous study (Chapter 2), leafy spurge flowers contained higher concentrations of CT than leaves and stems in June, although by August concentrations were higher in stems than leaves and flowers.

Condensed tannin concentrations also increased seasonally in all leafy spurge plant parts except for previously defoliated leaves. Condensed tannin concentrations in sericea (*Sericea lespedeza*) increase as the plant matures (Burns 1966, Cope et al. 1971); they are low when active growth begins, increase until mid-July, and then decrease to initial concentrations in mid-October (Cope et al. 1971). Condensed tannins also increase seasonally in birdsfoot trefoil (*Lotus corniculatus*) and big trefoil (*Lotus uliginosus*) (Chiquette et al. 1989, Lees et al. 1994). Early in the growing season, leafy spurge may allocate more nutrients to growth and reproduction than to producing tannins, which is associated with high metabolic costs (Skogsmyr and Fagerström 1992). Condensed tannins are carbon-based compounds; increases in the C/N ratio later in the growing season may provide more carbon for producing CT in leafy spurge.

Grasshopper Bioassays

For material collected in July and August, *M. sanguinipes* nymphs consuming leaves weighed more than nymphs consuming stems. In addition, percent mortality was lower for nymphs consuming leaves than for nymphs consuming stems, especially for August material. These responses may be explained by the higher CP and lower fiber content of leaves compared with stems, or the presence of CT. Condensed tannin

concentrations were negatively correlated with mean nymph weight. In August, concentrations of CT were higher in stems of leafy spurge than in July, which would explain the stronger effects on weights in August.

Other phenolics deter feeding in some acridids. Nearly all extracts obtained from 4 species in the Euphorbicaceae family deter feeding by *L. migratoria* (Bernays and Chapman 1977), an oligophagous acridid that feeds mainly on grasses (Bernays et al. 1976). The phenolic compound ellagic acid was identified in extracts; at concentrations of 2%, the compound reduced feeding of *L. migratoria* by 50%. Grasses generally contain relatively low concentrations of secondary plant compounds, particularly phenolics (Culvenor 1970). *Melanoplus sanguinipes* may be better adapted to phenolics in their diets, as they consume forbs and a wider variety of grasses than *L. migratoria* (Williams 1954, Mulkern et al. 1964). Mean weights of *M. sanguinipes* nymphs fed phenolics in a 5-day bioassay were lower than weights of nymphs on a control diet (Westcott et al. 1992). However, phenolics had no effect on nymph mortality. In our study, mortality tended to be higher for nymphs consuming stems than for nymphs consuming other plant parts. Stems contained higher concentrations of CT than other plant parts. However, percent nymph mortality was not correlated with CT concentration. Possibly, other factors in stems, such as low CP, high fiber content, or the presence of additional secondary compounds such as terpenoids affect nymph mortality.

We did not isolate secondary compounds such as the specific terpenoids or CT in leafy spurge to determine their individual and combined effects on *M. sanguinipes*. Synergistic effects of condensed tannins and other chemical defense compounds in leafy

spurge, including terpenoids, may affect the response of *M. sanguinipes* nymphs to the plant. Many plants contain a number of deterrent compounds, which, when combined often have stronger effects than individual compounds (Adams and Bernays 1978). Fourteen phenolic compounds, all deterrents at high concentrations, were tested on *L. migratoria*. Feeding on any of the individual compounds was not reduced, although when all 14 compounds were combined, feeding was greatly reduced. They concluded that the effects of individual chemicals at natural concentrations are often so slight that they do not reduce feeding, but the sum of individual effects leads to strong deterrence when they are combined. This may be a more flexible defense strategy for some plants. Plants may be able to produce low concentrations of costly compounds, that when in combination with other compounds, are more toxic or deterrent than higher concentrations of those costly compounds. In addition, specific compounds effect various herbivores differently. Plants that produce several secondary compounds may be protected from a wider variety of herbivores than plants that produce only one secondary compound.

Some important volatile compounds in leafy spurge may have been lost in the drying and grinding process. However, CT (Hemingway and Karchesy 1989) and most terpenoids (Gershenzon and Croteau 1991) are nonvolatile. Sheep rumen microorganisms produced similar amounts of gas when exposed to dry and wet leafy spurge, suggesting that volatile compounds, if any, have minimal effect (Thomas et al. 1994). However, dry matter disappearance of wet leafy spurge was lower than dry leafy spurge in their study.

Specific triterpenes are strong feeding deterrents (Butterworth and Morgan 1971, Haskell and Mordue 1969, Gill 1972). However, triterpenes occur frequently in grasses (Kulshreshtha et al. 1972), and probably are not important antifeedants to *M. locusta* (Bernays and Chapman 1977). Harley and Thorstseinson (1967) determined the effects of secondary compounds on a generalist grasshopper *M. bivittatus* (Say) which prefers forbs (Brooks 1958); the terpenoid saponin caused all nymphs to die before the adult stage was reached. Triterpenes are present in large concentrations in leafy spurge (Mahlberg personal comm., Evans and Kinghorn 1977). Because *M. sanguinipes* consume a variety of grasses and forbs, they may respond differently to triterpenes in their diet.

For plant material collected in August, increasing levels of undefoliated leafy spurge leaves increased mean grasshopper nymph weight, possibly because of the higher CP content of leafy spurge leaves compared to grass hay. On the other hand, increasing levels of leafy spurge flowers, which also had high CP content, decreased mean nymph weights. Condensed tannin concentrations of leaves and flowers were similar so they probably did not affect grasshopper nymph weights. Presumably, other secondary compounds, including terpenoids, in leafy spurge flowers decreased nymph weights.

Conclusions

Increasing levels of leafy spurge plant parts had no effect on grasshopper nymph mortality. However, mortality was higher for nymphs that consumed stems than for nymphs that consumed leaves or flowers. Condensed tannins concentrations were highest in stems in August; however, CT were not correlated with nymph mortality. Possibly,

other factors in stems, such as low CP, high fiber content, or the presence of additional secondary compounds such as terpenoids affect nymph mortality.

In July and August, ending weights were higher for nymphs that consumed leaves or flowers than for nymphs that consumed stems. In addition, ending weights were highest for nymphs that consumed leaves from previously defoliated shoots. Apparently, leaves from previously defoliated leafy spurge shoots do not contain higher concentrations of secondary compounds that adversely affect *M. sanguinipes* nymph weight and mortality, compared with leaves from undefoliated shoots.

The proportion of leafy spurge affected nymph weight only in August, but responses between plant parts were inconsistent. Generally, increasing levels of leaves of leafy spurge increased nymph weight. Negative associative effects were observed for stems, with 25 and 100% leafy spurge mixtures producing the highest nymph weights. Proportion of leafy spurge had no effect on nymph mortality.

Concentrations of CT in leafy spurge increased seasonally, and were higher in stems from previously defoliated shoots than in stems from undefoliated shoots. Condensed tannin concentrations were negatively correlated with grasshopper nymph weight and may play a role in feeding deterrence. For this study only 1 site was sampled; thus, these effects cannot be inferred to all leafy spurge populations. However, we observed similar trends in CT concentrations following defoliation on another field site (Chapter 2). In addition, we did not isolate secondary compounds, such as the specific terpenoids or CT in leafy spurge to determine their individual effects on *M. sanguinipes* nymph weight and mortality. Therefore, synergistic effects of condensed tannins and

other chemical defense compounds in leafy spurge, including terpenoids, may affect the overall response of *M. sanguinipes* nymphs to the plant.

CHAPTER 4

SUMMARY

Condensed tannins were present in leafy spurge leaves, flowers, and stems collected in 1994 and 1995. For all plant parts except previously defoliated leaves, CT concentrations increased throughout the growing season. In addition, CT concentrations were highest in leafy spurge stems, particularly in stems from previously defoliated shoots. These effects cannot be inferred to all leafy spurge populations. However, for material collected from both sites, we observed similar trends in CT concentrations throughout the growing season and following defoliation. Overall, CT concentrations were lower for material collected in 1995 compared with material collected in 1994. Condensed tannin concentrations within plant species vary by genotype (Barry and Manley 1984), nutrient availability (Gershenzon 1984), and temperature regime (Baldwin et al. 1987, Lees et al. 1994). The summer of 1994 was warmer and drier than the summer of 1995; CT concentrations in 1994 may have been high because of high-temperature stress (Lees et al. 1994). Alternatively, differences in nutrient availability on the 2 sites may have affected CT concentrations. Plants on sites with low soil fertility tend to produce higher concentrations of CT than those on sites with high soil fertility (Skogsmyr and Fagerström 1992). Considerable genotypic variability in leafy spurge (Rowe et al. 1993) may also account for these differences.

Increasing levels of leafy spurge leaves stimulated sheep rumen microbial activity and mass up to the 75% level, and increased *M. sanguinipes* nymph weight in August.

Therefore, relatively high levels of leafy spurge leaves are beneficial to sheep rumen microorganisms and migratory grasshopper nymphs. Increasing levels of leafy spurge flowers also stimulated microbial activity and mass, but tended to decrease grasshopper nymph weight. Possibly, phytochemicals are present in leafy spurge flowers that have no negative impacts on sheep rumen microorganisms, but decrease migratory nymph weight.

Grasshopper nymph mortality was not correlated with CT concentrations. *Melanoplus sanguinipes* consume a wide variety of forbs (Williams 1954), which generally contain much higher concentrations of secondary compounds than grasses. Possibly, *M. sanguinipes* have co-evolved with CT enough so that mortality does not increase when exposed to specific levels of CT.

Rumen microbial mass and activity, and grasshopper nymph weight and survivorship were negatively affected by leafy spurge stems. These responses correlate with high concentrations of CT in leafy spurge stems throughout the growing season. Additional qualities of stems, including low CP content, high fiber content, or the presence of additional secondary compounds such as terpenoids may also affect these responses. Synergistic effects of condensed tannins and other secondary defense compounds in leafy spurge probably determine the overall responses of sheep rumen microorganisms and migratory grasshoppers to the plant.

LITERATURE CITED

AOAC. 1984. Official methods of analysis (14th ed.). Association of Official Analytical Chemists. Washington, D.C.

Adams, C.M., and E.A. Bernays. 1978. The effect of combinations of deterrents on the feeding behaviour of *Locusta migratoria*. Ent. Exp. Appl. 23:101-109.

Adams, D.C., J.A. Pfister, R.E. Short, R.G. Cates, B.W. Knapp, and R.D. Wiedmeier. 1992. Pine needle effects on in vivo and in vitro digestibility of crested wheatgrass. J. Range Manage. 45:249-253.

Austin, P.J., L.A. Sucher, C.T. Robbins, and A.E. Hagerman. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. J. Chem. Ecol. 15:1335-1347.

Bae, H.D., T.A. McAllister, J. Yanke, K.J. Cheng, and A.D. Muir. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59:2132-2138.

Baldwin, I.T. 1988. Short-term damage-induced increases in tobacco alkaloids protect plants. Oecologia. 75:367-370.

Baldwin, I.T., J.C. Schultz, and D. Ward. 1987. Patterns and sources of leaf tannin variation in yellow birch (*Betula allegheniensis*) and sugar maple (*Acer saccharum*). J. Chem. Ecol. 13:1069-1078.

Bangsund, D.A., J.F. Baltezore, J.A. Leitch, and F.L. Leistritz. 1993. Economic impact of leafy spurge on wildlands in Montana, South Dakota, and Wyoming. North Dakota State Univ. Ag. Exp. Sta., Ag. Econ. Rpt. 304.

Barry, T.N. 1985. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. Br. J. Nutr. 54:211-217.

Barry, T.N., and S.J. Duncan. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. Br. J. Nutr. 51:485-491.

Barry, T.N., and T.R. Manley. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr. 51:493-504.

Barry, T.N., T.R. Manley, and S.J. Duncan. 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. Br. J. Nutr. 55:123-137.

Bartz, S., B. Landgraf, P.L. Fay, and K. Havstad. 1985. Leafy spurge (*Euphorbia esula*) as a forage component for ewes and lambs. SID Research Digest. Vol. 1, 2:39.

Bernays, E.A., and D.J. Chamberlain. 1980. A study of tolerance of ingested tannin in *Schistocerca gregaria.* J. Insect Physiol. 26:415-420.

Bernays, E.A., and R.F. Chapman. 1977. Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.). Ecol. Entomol. 2:1-18.

Bernays, E.A., R.F. MacDonald, and J.E.R. Salter. 1976. The degree of oligophagy in *Locusta migratoria* L. Ecol. Entomol. 1:223-230.

Bernays, E.A., D.J. Chamberlain, and P. McCarthy. 1980. The differential effects of ingested tannic acid on different species of Acridoidea. Entomol. Exp. Appl. 16:329-342.

Brooks, A.R. 1958. Acridoidea of Southern Alberta, Saskatchewan, and Manitoba (Orthoptera). Can. Entomol. Supp. 9. Vol 90.

Bryant, J.P., and P.J. Kuropat. 1980. Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. Annu. Rev. Ecol. and Syst. 11:261-285.

Bryant, J.P., F.S. Chapin, and D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.

Bryant, J.P., P.B. Reichardt, and T.B. Clausen. 1992. Chemically mediated interactions between woody plants and browsing mammals. J. Range Manage. 45:18-24.

Burns, R.E. 1966. Tannin in *Sericea lespedeza.* Ga. Agric. Exp. Stn. Bull. N.S. 164.

Burns, R.E. 1971. Methods for estimation of tannin in grain sorghum. Agron. J. 63:511-512.

Butler, L.G., J.C. Rogler, H. Mehanso, and D.M. Carlson. 1986. Dietary effects of tannins. *In:* A.R. Liss (ed.), Plant flavenoids in biology and medicine: biochemical, pharmacological, and structure-activity relationships. New York.

Butterworth, J.H., and E.D. Morgan. 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree, *Azadirachta indica.* J. Insect Physiol. 17:969-977.

Cassida, K.A., B.A. Barton, R.L. Hough, M.H. Wiedenhoeft, and K. Guillard. 1994. Feed intake and apparent digestibility of hay-supplemented brassica diets for lambs. J. Anim. Sci. 72:1623-1629.

Chapin, F.S., J.P. Bryant, and J.F. Fox. 1985. Lack of induced chemical defense in juvenile Alaskan woody plants in response to simulated browsing. Oecologia 67:457-459.

Chase, C.C. Jr., and C.A. Hibberd. 1987. Utilization of low-quality native grass hay by beef cows fed increasing quantities of corn grain. J. Anim. Sci. 65:557.

Chiquette, J., K.J. Cheng, L.M. Rode, and L.P. Milligan. 1989. Effect of tannin content in two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) on feed digestibility and rumen fluid composition in sheep. Can. J. Anim. Sci. 69:1031-1039.

Clausen, T.P., J.P. Bryant, and P.B. Reichardt. 1986. Defense of winter-dormant green alder against snowshoe hares. J. Chem. Ecol. 12:2117-2131.

Clausen, T.P., P.B. Reichardt, J.P. Bryant, R.A. Werner, and K. Post. 1989. Chemical model for short-term induction in quaking aspen (*Populus tremuloides*) foliage against herbivores. J. Chem. Ecol. 15:2335-2345.

Cooper, S.M., and N. Owen-Smith. 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. Oecologia. 67:142.

Cope, W.A., T.A. Bell, and W.W.G. Smart. 1971. Seasonal changes in an enzyme inhibitor and tannin content in *Sericea lespedeza.* Crop Sci. 11:893-895.

Cottee, P.K., E.A. Bernays, and A.J. Mordue. 1988. Comparisons of deterrency and toxicity of selected secondary plant compounds to an oligophagous and a polyphagous acridid. Entomol. Exp. Appl. 46:241-247.

Culvenor, C.C.J. 1970. Toxic plants - a re-evaluation. Search. 1:103-110.

Evans, F.J., and A.D. Kinghorn. 1977. A comparative phytochemical study of the diterpenes of some species of the genera *Euphorbia* and *Elaeophorbia* (*Euphorbicaceae*). Bot. J. Linn. Soc. 74:23-27 .

Evans, F.J., and C.J. Soper. 1978. The tigliane, daphnane and ingenane diterpenes, their chemistry, distribution and biological activities. A review. Lloydia 41:193-230.

Fay, P.K. 1991. Controlling leafy spurge with grazing animals. *In:* L.F. James, J.O. Evans, M.H. Ralphs and R.D. Child (eds.), Noxious range weeds. Westview Press, Boulder.

Feeny, P. 1976. Plant apparency and chemical defense. p. 1-40. *In:* J.W. Wallace, R.L. Mansell (eds.), Recent advances in phytochemistry: Vol. 10: Biochemical reactions between plants and insects. Plenum Press, New York.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *In:* G.N. Timmerman, C. Steelink, and F.A. Loewus (eds.), Recent advances in phytochemistry: Vol. 18: Phytochemical adaptations to stress. Plenum Press, New York.

Gershenzon, J., and R. Croteau. 1991. Terpenoids, 165-219. *In*: Rosenthal, G.A. and D.H. Janzen (eds.), Herbivores: their interactions with plant secondary metabolites. Academic Press, New York.

Gill, J.L. 1991. Evolution of statistical design and analysis of experiments. J. Dairy Sci. 64:1494-1519.

Gill, J.S. 1972. Studies on insect feeding deterrents with special reference to the fruit extracts of the neem tree, *Azadirachta indica*, A. Juss. Ph.D. thesis, University of London.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). ARS, USDA Agr. Handb. 379.

Harborne, J.B. 1986. Flavenoid patterns and phytogeography: the genus Rhododendron section Vireya. Phytochemistry 25:1641-1643.

Harley, K.L.S., and A.J. Thorsteinson. 1967. The influence of plant chemicals on the feeding behavior, development, and survival of the two-striped grasshopper, *Melanoplus bivittatus* (Say), Acrididae: Orthoptera. Can J. Zool. 45:305-319.

Hart, S.P. 1987. Associative effects of sorghum silage and sorghum grain diets. J. Anim. Sci. 64:1779-1789.

Haskell, P.T., and A.J. Mordue. 1969. The role of mouthpart receptors in the feeding behavior of *Schistocerca gregaria*. Entomol. Exp. Appl. 12:591-610.

Hassan, A.A., H.D. Woody, and A.W. Young. 1988. Associative effects of mixed diets in sheep. Trans. Ill. Acad. Sci. 81:1-8.

Hemingway, R.J., and J. Karchesy. 1989. Chemistry and significance of condensed tannins. Plenum Press, New York.

Hinks, C.F., M.T. Cheeseman, M.A. Erlandson, O. Olfert, and N.D. Westcott. 1991. The effects of kochia, wheat and oats on digestive proteinases and the protein economy of adult grashoppers, *Melanoplus sanguinipes*. J. Insect Physiol. 37:417-430.

Hinks, C.F., O.O. Olfert, and N.D. Westcott. 1987. Screening cereal cultivars for resistance to early-instar grasshoppers. J. Agric. Entomol. 4:315-319.

Jandel Scientific. 1993. SigmaStat user's manual. San Rafael, CA.

Johnson, A., and R.W. Peake. 1960. Effect of selective grazing by sheep on the control of leafy spurge (*Euphorbia esula* L.). J. Range Manage. 13:192-195.

Jones, W.T., and J.L. Mangan. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) With fraction 1 leaf protein and with submaxillary muco-protein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28:126-136.

Khan, M.B., and J.B. Harborne. 1990. Induced alkaloid defense in *Atropa acuminata* in response to mechanical and herbivore leaf damage. Chemoecology 1:77-84.

Khazaal, K., X. Markantonatos, A. Nastis, and E.R. Ørskov. 1992. Changes with maturity in fibre composition and levels of extractable polyphenols in Greek browse: effects on *in vitro* gas production and *in sacco* dry matter degradation. J. Sci. Food Agric. 63:237-244.

Kulshreshtha, M.J., D.K. Kulshreshtha, and R.P. Rastogi. 1972. The triterpenoids. Phytochemistry 11:2369-2381.

Kumar, R., and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447-453.

Lacey, C.A., P.L. Fay, R.G. Lym, C.G. Messersmith, B. Maxwell, and H.P. Alley. 1985. The distribution, biology and control of leafy spurge. Montana State Univ. Coop. Ext. Ser. Circ. 309.

Lajeunesse, S., R. Sheley, R.G. Lym, D. Cooksey, C. Duncan, J. Lacey, N. Rees, and M. Ferrell. 1995. Leafy spurge: biology, ecology and management. Montana State Univ. Coop. Ext. Ser.

Lambert, M.G., S.M. Abrams, H.W. Harpster, and G.A. Jung. 1987. Effect of hay substitution on intake and digestibility of forage rape (*Brassica napus*) fed to lambs. J. Anim. Sci. 65:1639.

Landgraf, B.K., P.L. Fay, and K.M. Havstad. 1984. Utilization of leafy spurge (*Euphorbia esula*) by sheep. Weed Sci. 32:348-352.

Lees, G.L., C.F. Hinks, and N.H. Suttill. 1994. Effect of high temperature on condensed tannin accumulation in leaf tissues of big trefoil (*Lotus uliginosus* Schkuhr). J. Sci. Food Agric. 65:415-421.

Lingle, S.E., and J.C. Suttle. 1985. A model system for the study of 2,4-D translocation in leafy spurge. Can. J. Plant Sci. 65:369-377.

Lym, R.G. 1992. Absorption and translocation of foliar-applied sulfometuron in leafy spurge (*Euphorbia esula*). Weed Sci. 40:477-481.

Lym, R.G., and D.R. Kirby. 1987. Cattle foraging behavior in leafy spurge (*Euphorbia esula*) infested rangeland. Weed Tech. 1:314-318.

Lym, R.G., and K.D. Moxness. 1989. Absorption, translocation and metabolism of picloram and 2,4-D in leafy spurge (*Euphorbia esula*). Weed Sci. 37(4):498-502.

Lym, R.G., C.G. Messersmith, and D.E. Peterson. 1988. Leafy spurge identification and control. North Dakota Ext. Serv. Cir. W-765.

Macdearmid, A., P.E.V. Williams, and G.M. Innes. 1983. Turnips with chemically-treated straw for beef production. 1. The effects on the growth of steers. Anim. Prod. 37:97-104.

McDougall, E.I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43:99.

Mehanso, H., T.N. Asquith, L.G. Butler, J.C. Rogler, and D.M. Carlson. 1992. Tannin-mediated induction of proline-rich protein synthesis. J. Agr. Food Chem. 40:93-97.

Mihaliak, C.A., and D.E. Lincoln. 1989. Plant biomass partitioning and chemical defense: response to defoliation and nitrate limitation. Oecologia 80:122-126.

Moe, P.W. 1979. Associated effects of feedstuffs. Pacific Northwest Anim. Nutr. Conf. p. 177.

Montana State University and USDA. 1982. Soils of Montana. Montana Agr. Expt. Sta. Bull. 744.

Mulkern, G.B., D.R. Toczek, and M.A. Brusven. 1964. Biology and ecology of North Dakota grasshoppers II. Food habits and preference of grasshoppers associated with the sand hills prairie. N. Dakota Agr. Expt. Sta. Res. Rep. 11:59.

Nagy, J.G., and R.P. Tengerdy. 1968. Antibacterial action of essential oils of *Artemisia* as an ecological factor. II. Antibacterial action of the volatile oils of *Artemisia tridentata* (big sagebrush) on bacteria from the rumen of mule deer. Appl. Microbiol. 16:441-444.

Nagy, J.G., H.W. Steinhoff, and G.M. Ward. 1964. Effect of essential oils of sagebrush on deer rumen microbial function. J. Wildl. Manage. 28:785-790.

Oh, H.K., T. Sakai, M.B. Jones, and W.M. Longhurst. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15:777-783.

Olfert, O., C.F. Hinks, and N.D. Westcott. 1990. Resistance of *Kochia scoparia* (L.) to feeding damage by grasshoppers (Orthoptera: Acrididae) and other herbivorous insects. J. Econ. Ent. 88:2421-2426.

Perevolotsky, A. 1994. Tannins in Mediterranean woodland species: lack of response to browsing and thinning. Oikos. 71:333-340.

Price, M.L., S. Van Scoyoc, and L.G. Butler. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26:1214-1218.

Provenza, F.D., and J.C. Malechek. 1984. Diet selection by domestic goats in relation to blackbrush twig chemistry. J. Appl. Ecol. 21:831-841.

Ralphs, M.H., J.D. Olsen, J.A. Pfister, and G.D. Manners. 1988. Plant-animal interactions in larkspur poisoning in cattle. J. Anim. Sci. 66:2334-2342.

Raju, M.V.S. 1985. Morphology and anatomy of leafy spurge, p. 26-41. *In:* A.K. Watson (ed.), Leafy spurge. Weed Science Society of America. Champaign, Ill.

Raubenheimer, D., and S.J. Simpson. 1990. The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behaviour of larval *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal). I. Short-term studies. Physiol. Ent. 15:219-233.

Redak, R.A., and J.L. Capinera. 1994. Changes in western wheatgrass foliage quality following defoliation: consequences for a graminivorous grasshopper. Oecologia 100:80-88.

Reichardt, P.B., J.P. Bryant, T.P. Clausen, and G.D. Wieland. 1984. Defense of winter-dormant Alaska paper birch against snowshoe hares. Oecologia 65:58-69.

Reichardt, P.B., J.P. Bryant, B.R. Mattes, T.P Clausen, and F.S. Chapin, III. 1990. The winter chemical defense of balsam poplar against showshoe hares. J. Chem. Ecol. 16:1941-1960.

Reichardt, P.B., T.P. Clausen, and J.P. Bryant. 1987. Plant secondary metabolites as feeding deterrents to vertebrate herbivores, p. 37-42. *In:* F.D. Provenza, J.T. Flinders and E.D. McArthur (eds.), Proc. Symp. on Plant-Herbivore Interactions. USDA Forest Serv. Gen. Tech. Rep. INT-222, Ogden/Intermountain Res. Sta.

Reilly, W., and K.R. Kaufman. 1979. The social and economic impact of leafy spurge in Montana, p.21-24. *In:* Proc. Leafy Spurge Symp. N.D. State Univ. Coop. Ext. Serv., Fargo, N.D., unnumbered publication.

Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores, p. 3-54. *In:* Rosenthal, G.A. and D.H. Janzen (eds.), Herbivores: their interactions with secondary plant metabolites. Academic Press, New York.

Robbins, C.T., S. Mole, A.E. Hagerman, and T.A. Hanley. 1987. Role of tannins in defending plants against ruminants: reduction in dry matter digestion? Ecology. 68:1606-1615.

Rowe, M.L., S.J. Nissen, P.J. Lee, and R.A. Masters. 1993. Genetic diversity of the leafy spurge chloroplast genome. Proc. Great Plains Agr. Council. Leafy Spurge Task Force Symp. Silvercrest, CO.

SAS. 1987. SAS User's Guide: Statistics. SAS Instl, Indl, Cary, NC.

Schildknecht, H. 1981. Irritant and defense substances of higher plants - a chemical herbarium. Agnew. Chem. Int. Ed. Engl. 20:164-184.

Sedivec, K.K., and R.P. Maine. 1993. Biological control of leafy spurge using angora goats. Abstr. No. 230. *In:* Abstr. 46th Annu. Meeting Soc. of Range Manage. Albuquerque, N.M.

Skogsmyr, I., and T. Fagerström. 1992. The cost of anti-herbivory defence: an evaluation of some ecological and physiological factors. Oikos 64: 451-457.

Smart, W.W.G. Jr., T.A. Bell, N.W. Stanley, and W.A. Cope. 1961. Inhibition of rumen cellulase by an extract from sericea forage. J. Dairy Sci. 44:1945-1946.

Spilatro, S.R., and P.G. Mahlberg. 1986. Latex and laticifer starch content of developing leaves of *Euphorbia pulcherrima*. Amer. J. Bot. 73:1312-1318.

Terrill, T.H., A.M. Rowan, G.B. Douglas, and T.N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58:321-329.

Thomas, V.M., C.K. Clark, R.W. Kott, and B. Olson. 1994. Influence of leafy spurge on ruminal digestion and metabolism and blood metabolite profiles in sheep. Sheep and Goat Res. J. 10:168-172.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.

Upadhyay, R.R., F. Bakhtavar, M. Ghaisarzadeh, and J. Tilabi. 1978. Cocarcinogenic and irritant factors on *Euphorbia esula* L. latex. Tumori 64:99-102.

USDA 1979. Soil survey of Fergus County, Montana. Montana Agr. Exp. Sta.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O & B Books, Corvallis, Ore.

Walker, J., and S. Kronberg. 1992. Preference of leafy spurge by sheep compared to goats, p. 1-2. *In:* Abstr. 45th Annu. Meeting Soc. of Range Manage. Spokane, Washington.

Walters, T., and H.A. Stafford. 1984. Variability in accumulation of proanthocyanidins (condensed tannins) in needles of douglas-fir (*Pseudotsuga menziesii*) following long-term budworm defoliation. J. Chem. Ecol. 10:1469-1476.

Westcott, N.D., C.F. Hinks, and O. Olfert. 1992. Dietary effects of secondary plant compounds on nymphs of *Melanoplus sanguinipes* (Orthoptera: Acrididae). Ann. Entomol. Soc. Am. 304-309.

Williams, L.H. 1954. The feeding habits and food preferences of Acrididae and the factors which determine them. Trans. R. Entomol. Soc. London 105:423-454.

Williams, P.E.V., A. Macdearmid, G.M. Innes, and A. Brewer. 1983. Turnips with chemically-treated straw for beef production. 2. Effect of turnips on the degradability of straw in the rumen [Steers]. Anim. Prod. 37:189-194.

Yokoyama, M.T., and K.A. Johnson. 1988. Microbiology of the rumen and intestine, p. 125-144. *In:* D.C. Church (ed.), The ruminant animal: digestive physiology and nutrition. Waveland Press, Inc., N.J.

Zinn, R.A., and F.N. Owens. 1986. A rapid procedure for purine measurements and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157.