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

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Joanna L. Roberts

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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.

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Date 4/11/96

Dedicated in memory of Dr. Verl M. Thomas.

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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.

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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.

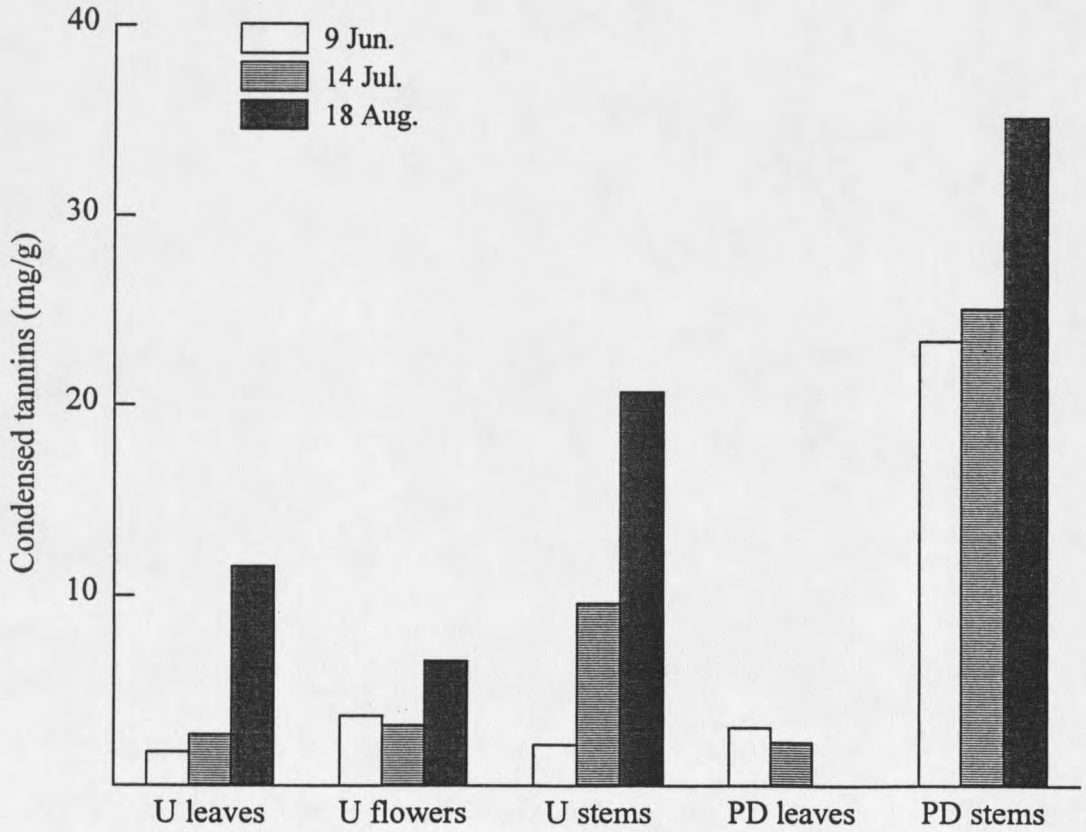
Plant Part	CP	EE	NDF	ADF	ADL
19 May 1994					
I Leaves	22.68	4.18	18.56	15.21	2.94
I Flowers	25.78	5.02	16.98	14.55	3.49
I Stems	9.38	3.03	48.01	40.68	5.96
9 Jun. 1994					
U Leaves	16.59	4.73	18.45	16.87	3.70
U Flowers	17.67	5.28	20.74	18.41	4.73
U Stems	4.85	2.60	63.65	53.64	9.70
PD Leaves	22.22	4.12	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.



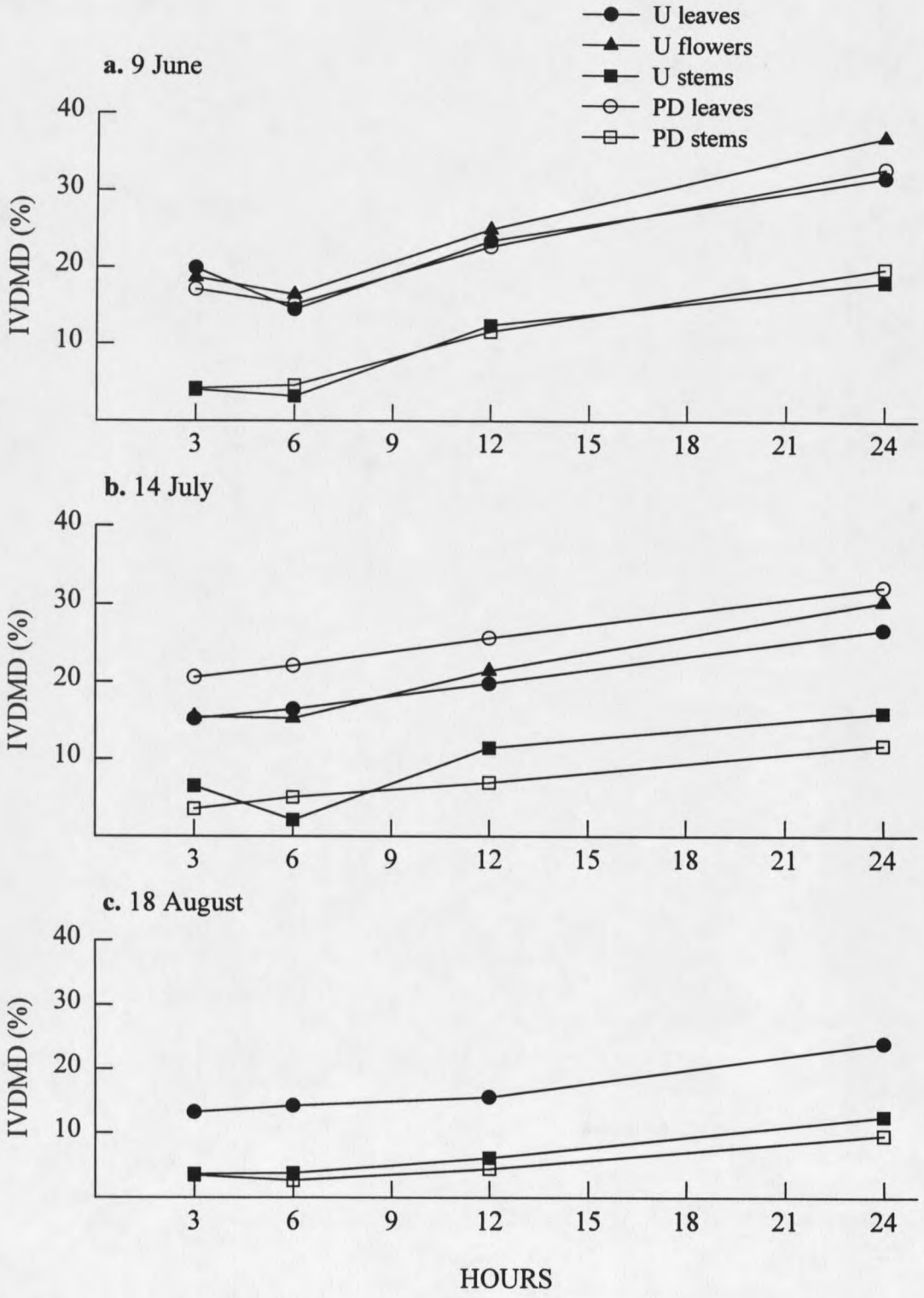
Conventional In Vitro Trial

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.