FOLIAR PHENOLICS OF NEBRASKA SANDHILLS
PRAIRIE GRAMINOIDS: BETWEEN-YEARS, SEASONAL, AND INTERSPECIFIC VARIATION

SIMON MOLE* and ANTHONY JOERN

School of Biological Sciences
University of Nebraska-Lincoln
Lincoln, Nebraska 68588-0118

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Abstract—Because of their potential as antiherbivore defenses, plant pheno-
olics elicit considerable attention. We made quantitative and qualitative anal-
yses of phenolics, alkaloids, cyanogenic glycosides, and saponins in the
dominant graminoids of a Nebraska Sandhills prairie. We examined the foli-
age of seven species: Agropyron smithii Rydb., Andropogon hallii Hack., Andro-
pogon scoparius Michx., Bouteloua gracilis (H.B.K) Lag. ex Griffiths, Cal-
amovilfa longifolia (Hook.) Scribn., Carex heliophila Mack., and Stipa comata
Trin & Rupr. Their leaves contain low levels of phenolics that vary sig-
ificantly among species. A more detailed examination of the three species with
the highest levels of phenolics showed among-year, seasonal, and spatial
heterogeneity in the levels of total phenolics. In all seven species, the majority
of the specific phenolics present have the chromatographic properties of
phenylpropanoids and are likely to be present as sugar-linked derivatives such
as free glycosides or cell wall-bound phenolics. These species do not contain
condensed tannins. The absence of other common kinds of secondary metab-
olites indicates that these graminoids are unlikely to have significant chemical
defenses, at least in terms of substances likely to be active against mammalian
herbivores. In this, they exemplify the situation thought typical for prairie
graminoids.

Key Words—Phenolics, seasonal variation, graminoids.

INTRODUCTION

Plant phenolics have excited considerable attention because of their potential
role as allelochemicals. In this role they may function against herbivores, plant
*To whom correspondence should be addressed; e-mail: simonmole@unl.edu

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tively, and it is important to note that the hydrolysis was performed with cell-
wall material present so as to release phenolics bonded (esterified) to it. Each 
fraction was partitioned against diethyl ether to give an ether-extractable frac-
tion. The remaining aqueous layer was then partitioned against ethyl acetate 
and then again against amyl alcohol to give ethyl acetate and amyl alcohol fractions, 
respectively. The remaining aqueous fractions were then lyophilized so that the 
phenolics contained in them could be concentrated. This was achieved by dis-
solving the freeze-dried residue in a small quantity of 50% methanol. Organic 
solvent was removed from the other fractions by evaporation under a stream of 
nitrogen gas. Small volumes of these concentrated fractions were applied to 
TLC systems for side-by-side analyses of samples from the seven different 
species.

TLC solvent systems are numbered for reference below. Those used with 
silica gel plates (Silica gel/F, Sigma Chemical Co.) were: (1) acetic acid-
chloroform (1:9) and (2) ethyl acetate-benzene (9:11); TLC solvent systems 
used with cellulose plates (Sigma Chemical Co.) were: (3) 100% water, (4) 
butanol–ethanol–water (4:1:2.2), (5) butanol–acetic acid–water (4:1:5, upper 
layer used), and (6) "forestal," which is hydrochloric acid–acetic acid–water 
(3:30:10). Phenolics were detected on the plates by examinations under short-
and longwave UV light, with and without fuming the plates with ammonia. 
Plates were also sprayed with the Prussian blue reagents (Price and Butler, 1977) 
so as to detect substances contributing to the total phenolics assay. Plates de-
veloped for simple phenolics were also sprayed with 10% (w/v) vanillin in hydro-
chloric acid as an additional detection reagent (Harborne, 1984).

Analyses made for nonphenolic secondary metabolites involved testing fresh 
material in the field for cyanogenesis early in the 1989 field season (May). This 
was done by enclosing samples of crushed leaves with Feigl-Anger test paper 
(Fieg and Anger, 1966). In the lab, aggregate samples constructed like those 
for the phenolics analyses (see above) were also tested for the presence of 
alkaloids. This followed the classic technique of extraction into aqueous acid 
(1 M HCl) followed by making the extract alkalizing extract with ammonia and 
then partitioning the alkaloid into chloroform. Concentrated samples of this 
extract were applied to TLC plates (Silica gel G, solvent: methanol–ammonia, 
200:3), and both the Dragendorff and the iodoplatinate reagents were used for 
detection. A more specific search for indole alkaloids was made using the tech-
niques of Mulvena and Slaytor (1982). Finally, a test for saponins was made 

Statistical analyses were performed using DOS SYSTAT 5.0. Means pre-
sented in the text are followed by standard errors in parentheses. The analyses 
of covariance presented in Tables 1 and 2 each have Julian date as the covariate 
and total phenolics content as the dependent variable. Kruskal-Wallis ANOVAs 
used to establish rank orderings of species mean phenolic levels within years 
were applied factorially to all pairwise species comparisons; after first establish-
ing an overall species effect for each year.

RESULTS

Temporal and Species-Specific Responses. On the basis of an initial study, 
we selected three species with relatively high levels of extractable phenolics for 
an intensive study: Andropogon hallii, Andropogon scoparius, and Carex helio-
phila. Table 1 presents a statistical analysis of the total phenolics contents 
of samples of these species collected from the valley site over the period 1985– 
1992. From this it can be seen that there are both species-specific differences 
in total phenolics content and among-year differences. Julian date was used as 
a covariate in the analysis because phenolics levels show some tendency to 
increase during the growing season (see below).

Over this seven-year period, A. hallii, A. scoparius, and C. heliophila 
exhibited mean levels of 1.31% (0.020), 1.37% (0.020), and 0.96% (0.021) 
total phenolics, respectively. C. heliophila contained significantly less phenolic 
material relative to the other two species (P < 0.001), which do not differ 
significantly from each other in total phenolic content. These statistical tests 
result from pairwise comparisons of means made subsequent to the ANCOVA 
presented in Table 1 (Bonferroni procedure).

Figure 1 shows the levels of phenolics in these three species for the different 
years of the study. Both A. hallii and C. heliophila show little variation, par-
ticularly relative to each other, while A. scoparius is variable. By inspection 
of these data, the most frequent pattern seems to be for 1987, 1988, and 1990, 
where the rank order of the species means for phenolic content is A. scoparius > 
A. hallii > C. heliophila. This result is statistically significant for each of 
these years (P < 0.002; Kruskal-Wallis ANOVA). While the pattern is similar 
for 1985 and 1991, the means for the two Andropogon species cannot be dis-

Table 1. ANCOVA for Total Phenolics Content of Three Dominant Sandhills 
Graminoids

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MSa</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>6</td>
<td>2.250</td>
<td>0.001</td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>8.755</td>
<td>0.001</td>
</tr>
<tr>
<td>Species * Year</td>
<td>12</td>
<td>0.941</td>
<td>0.001</td>
</tr>
<tr>
<td>Julian date</td>
<td>1</td>
<td>10.083</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>659</td>
<td>0.080</td>
<td></td>
</tr>
</tbody>
</table>

*MS = mean squares.
ridge sites have more phenolics than samples collected from the valley location (P < 0.001).

**UV(B) Absorbance.** Scans of the UV absorbance of the extracts in the UV(B) range showed no sharp peaks of absorbance in any species, instead the spectra ranged from being relatively flat to cases where there was a decline in absorbance by about 50% from 280 nm to 320 nm (e.g., *Agropyron smithii*, * Bouteloua gracilis*). Those species with high total phenolics levels had high absorbances, as would be expected given the UV-absorbing nature of phenolics. Mean absorbances at 280 nm were: *Andropogon hallii*, 34.3 (0.26); *Andropogon scoparius*, 33.26 (0.41); and *Carex heliophila*, 27.2 (1.00). For the species with lower levels of phenolics, the absorbances were: *Agropyron smithii*, 14.6 (0.89); *Bouteloua gracilis*, 6.5 (0.37); *Calamovilfa longifolia*, 14.9 (1.03); and *Sipta comata*, 10.6 (0.69).

**Chromatographic Analyses.** Our goal in chromatographic analyses was to establish whether there were qualitative differences in phenolics between species, rather than to isolate and characterize specific phenolic metabolites. All seven species of graminoids from the site were analyzed (data presented below).

Unhydrolyzed aqueous extracts can be expected to contain substances as they are encountered in the plant, so we focused on results for these. The ether fractions from these extracts, chromatographed on TLC systems 1 and 2, were devoid of any UV-absorbing substances that reacted with Prussian blue reagents, providing no evidence for simple phenolics such as phenolic acids. Negative results with the vanillin reagent spray did not indicate any resorcinol or phloroglucinol derivatives in this extract. Analyses of the ethyl acetate fractions did reveal evidence for several phenolics. The forestal TLC system (6) revealed a substance that appeared bright yellow under UV at RT = 0.83 in both *C. heliophila* and *C. longifolia*. This reacted with the Prussian blue reagents and is likely to be a flavonoid (e.g., flavonol). TLC using solvent systems 4 and 5 enabled the detection of several substances, all of which reacted positively with Prussian blue reagents and all of which appeared various shades of blue under UV light. In system 4, such substances were detected in *A. scoparius* (RT = 0.72), *C. heliophila* (RT = 0.61) and *C. longifolia* (RT = 0.66, 0.71, 0.83). In system 5, substances were detected in *A. smithii*, (RT = 0.57), *A. hallii* (RT = 0.66), and *C. heliophila* (RT = 0.60). These characteristics suggest that these phenolics are most likely to be phenylpropanoids. The amyl alcohol extracts were colorless, and the only fraction remaining for analysis was the aqueous one. In this fraction numerous substances with the above characteristics of phenyl propanoids were found. For TLC in system 5, these were detected in *A. smithii*, (RT = 0.30, 0.46, 0.54, 0.61), *A. hallii* (RT = 0.46, 0.54, 0.61), *A. scoparius* (RT = 0.46, 0.54, 0.61), *C. heliophila* (RT = 0.61) and *B. gracilis* (RT = 0.46), *C. longifolia* (RT = 0.46, 0.54, 0.61), *C. heliophila* (RT = 0.61) and *S. comata* (RT = 0.48, 0.61). For TLC in system 3, two likely phenyl propanoids were detected, one at RT = 0.13 in *C. longifolia* and one at RT = 0.64 in *C. longifolia*, *C. heliophila*, and *S. comata*.

The analysis of the hydrolyzed samples was considerably more complex than the above. The main difference from the unhydrolyzed samples was that most substances were detected in the TLC analyses of the ether and ethyl acetate fractions, while the remaining aqueous fraction was essentially devoid of phenolics. None of the substances found in the aqueous fraction before hydrolysis remained after hydrolysis. In each TLC system used with the ether or ethyl acetate fractions, each species gave a chromatogram that typically had two to four substances that were blue, blue-green, or mauve under UV light or UV plus ammonia and that all reacted with the Prussian blue reagents. Many of these were common to several species, and their absence from these fractions generated from the unhydrolyzed extracts suggests that they are normally present as free glycosides or bound to the cell wall via O-glycosidic linkages. There was no evidence for the presence of substantial quantities of flavonoids in these species except for *A. hallii* and *A. scoparius*, which both yielded strongly orange-brown amyl alcohol fractions. TLC of these fractions in forestal produced a brown smear suggestive of phlobaphenes, but with a faint yellow band at an Rf indicative of the 3-deoxy anthocyanidin apigenin. There was no evidence of cyanidin or any other anthocyanidin in the amyl alcohol fraction of any species.

Additional samples of all seven species were tested for alkaloids, cyanogenesis, and the presence of saponins. The results for these tests were negative in every case.

**DISCUSSION**

The results indicating that these Sandhills graminoids have total phenolics contents of approximately 1% are within the expected range for graminoids, which typically have lower levels of phenolics than dicotyledonous plants. Waterman et al. (1983) report total phenolics contents of 1.38% and 1.23% for Carex bequaertii (Cyperaceae) and Arundinaria alpina (Gramineae), respectively. These species were placed at the extreme low end of the range of total phenolics contents measured in a study of leaves from 18 other, exclusively dicotyledonous, species sampled from a montane flor in Africa. At the upper limit of the range was a species with 17% total phenolics content. Most surveys of plant phenolics are concerned with dicotyledonous plants, where it is usual for the range of total phenolics contents to be between 0% and 20%, with few species containing 1% or less phenolic material (Gartlan et al., 1980; Janzen and Waterman, 1984; Marks et al., 1988). It is also typical for plants with high total phenolics contents to have either condensed or hydrolyzable tannins. The species in this study are typical graminoids in that they have the relatively low
total phenolics contents expected of species that do not possess tannins in any significant quantity.

Our results indicating between-years variation in phenolics have important implications, even though the absolute levels of phenolics are low. In biological terms, plant phenolics may still be important as guides to host-plant selection, such as by grasshoppers. Herbivores accepting or rejecting material based on threshold levels of phenolics can be expected to make different selections in different years given these results. In terms of the practical problems of plant analysis, interspecific comparisons of total phenolics made on the basis of a single year’s sampling may not be replicable in subsequent years, even on a relative basis.

In the only other long-term study to examine between-years variation in a graminoid, Jonasson et al. (1986) also found significant between-years variation in the total phenolics content of *Eriophorum vaginatum* (Cyperaceae) in an eight-year study. In this species from Swedish Lapland, yearly mean total phenolics levels ranged between 3.9% and 5.4%. Unlike the species in the present study, *E. vaginatum* has substantially higher levels of phenolics, and it also contains condensed tannins (Jonasson et al., 1986). Indeed, their study indicates that virtually all the phenolics present were condensed tannins.

Jonasson et al. (1986) considered the likely factors causing between-years variation in their system. They could find no evidence that cyclic variation in microtine rodent (herbivore) population levels was important, while in our system there has not been any exceptional outbreak of grasshoppers during our study. Instead of herbivory, Jonasson et al. (1986) considered climate to be the dominant factor, observing high levels of phenolics in warm years. Plant growth is unlikely to be strongly limited by cool temperatures in the Nebraska Sandhills, but rainfall may be a limiting factor. In 1989, when phenolics levels were particularly low, rainfall was the lowest recorded since on-site recording began (1987). While the relationship between water deficit and phenolics metabolism may be nonlinear, Horner (1990) has shown a decline in tannin production at extreme water deficit. Further and longer-term studies might well uncover more definitive correlations between climatic effects and total phenolics production in the Sandhills system.

The results showing an increasing trend for total phenolics with time, during the growing season, are uncontroversial for dicotyledonous plants (Feeny, 1968; Mauffette and Oechel, 1989; Tempel, 1981). The only available data for a graminoid is from a second study of *E. vaginatum* by Chapin et al. (1986), working in the Alaskan Tundra. Here total phenolics levels for the leaves were lower than those from the Swedish site, with mean levels in the range 2.5–3.5%. No significant seasonal variation was seen during three years of study. We do find significant variation (Figure 2), but more studies will be required to obtain a general result for graminoids as distinct from dicots.

The results indicating variation between valley, slope, and ridge sites demonstrate significant spatial heterogeneity among conspecifics of all three species. There is evidence that plant-water relations vary along transects up sandhills (Barnes and Harrison, 1982) and that a plant water deficit is a factor in plant phenolics metabolism (Horner, 1990). This suggests the potential for drainage and topography to influence plant phenolic content, but further work, including replication on other dunes, is needed to establish this as a factor. A potential and unexplored confounding source of variation is spatial heterogeneity in soil mineral nutrients, particularly as soil nitrogen may be higher in valley sites (unpublished data).

The levels of phenolics in the Sandhills graminoids analyzed here do not reach the levels representative of plants with "quantitative" defenses against grazing (see above). This raises the question of other possible functions that they may have in these species. An early suggestion by Rhoads and Cates (1976) was that phenolics acted as a screen against UV light, but this suggestion was based in a study of creosote bush, where the phenolics are present as an extracellular resin on the surface of leaves. For these grasses, the phenolics are present internally in the leaf, most likely in cell vacuoles, which will make them less able to screen out UV light. There is also a fivefold range in the UV absorbances of the extractable phenolics found in this study. Furthermore, dicotyledonous plants may have an order of magnitude or more phenolics than the most phenol-rich Sandhills graminoid. Given these observations, it would seem that at least some of these graminoids are either poorly protected against UV relative to other species or that this function is accomplished some other way than by the possession of phenolics.

The particular kinds of phenolics present are unexceptional. The absence of free simple phenolics, as seen from the TLC analysis of the unhydrolyzed extract, is typical (Fahey and Jung, 1989). Hydrolyzable tannins have never been found in grasses or sedges, and the absence of condensed tannins is indicated here by the failure to find the anthocyanins (cyanidin and delphinidin) in the amyl alcohol fractions of the hydrolyzed extract (Harborne, 1984). In addition to finding no chemical evidence for tannins, we also failed to find any evidence of protein-precipitating activity in simple qualitative tests made on aqueous extracts (Mole and Joern, unpublished observations). Most of the phenolics located on TLC plates were phenyl propanoids or their derivatives. Substances isolated from the unhydrolyzed extract in the remaining aqueous fraction were most likely to have been phenylpropanoid glycosides. The substantial generation of ether- and ethyl acetate-soluble phenolics after hydrolysis suggests the breakdown of these glycosides and, perhaps, the additional release of cell wall-bound phenolics by hydrolysis. The presence of such substances is usual for grasses, and in general they present no antinutritional problems to ruminants.
(Fahey and Jung, 1989). Virtually nothing is known about the interaction of such substances with insects such as graminivorous grasshoppers.

There is sufficient chemical variety in the phenolics found in the unhydrolyzed extracts that an insect able to distinguish these from each other would have sufficient information to discriminate between these species without reference to other information: i.e., considering all the TLC analyses, each species contains a unique variety of phenolics in unhydrolyzed extracts.

Also typical for grasses and sedges is the lack of other kinds of allelochemicals such as alkaloids, saponins, and cyanogenic glycosides. We tested for cyanogenic glycosides early in the season when they are known to be present in the young growth of some grasses (Haskins and Gorz, 1988). Arapahoe Prairie is likely to be a particularly nitrogen-poor system (Burke et al., 1991), and this may be a factor in their absence and the absence of alkaloids, all of which contain nitrogen. Several grass species have been found to contain endophytic alkaloids in recent years, but these seem to be more restricted to C3 species found in relatively moist habitats in states east of Nebraska (Hemken and Bush, 1989). In addition, the species surveyed here are not in genera where indole alkaloids are to be expected (Corcuera, 1989), and so the failure to detect alkaloids comes as no real surprise.

In summary, the species on which this study has focused are the dominant graminoids at a Nebraska Sandhills prairie site. Their leaves have low levels of phenolics, which are variable among years and between species. There is also some evidence for seasonal and spatial heterogeneity in levels of total phenolics. The specific phenolics present do not include condensed tannins and are likely to be phenylpropanoids, particularly sugar-linked derivatives such as free glycosides or cell wall-bound phenolics. This and the absence of other common kinds of secondary metabolites indicate that these graminoids are unlikely to have significant chemical defenses, at least in terms of substances likely to be active against mammalian herbivores. In this they exemplify the situation thought typical for such species, prior to recent research drawing attention to exceptional toxicities such as those for tall fescue or Phalaris alkaloids. We do not in any way contradict this work, but do suggest that not all the grasses in grassland systems are full of high levels of secondary metabolites awaiting discovery.

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REFERENCES


EFFECTS OF SOME LEAF-EMITTED VOLATILE COMPOUNDS ON APHID POPULATION INCREASE


Departments of Agronomy, Entomology, and Horticulture University of Kentucky Lexington, Kentucky 40546

Crops Research Laboratory USDA-ARS Oxford, North Carolina

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Abstract—A role of some volatile compounds produced by plant tissues may be as defensive molecules against various pests, including aphids. Volatile six-carbon compounds derived in plant tissue from polyunsaturated fatty acids via lipoygenase/hydroperoxide lyase reduced tobacco aphid fecundity at certain concentrations when added to headspace vapor to which aphids were exposed. Both C6 aldehydes and alcohols were effective, with the alcohols having greater activity. (Z)-3-Hexenyl acetate at levels in the headspace similar to those of the alcohols and aldehydes did not reduce aphid fecundity. A 6-h exposure period to the C6 aldehydes and alcohols was needed for maximum effect on the aphids feeding on tobacco leaves. Analysis of the direct versus indirect effects of these compounds indicates that the volatile aldehydes had both direct effects on aphid fecundity and indirect effects due to induced changes in the leaves upon which the aphids were feeding, while only indirect effects were observed for the alcohols. Tomato leaves have the capacity to produce volatile compounds at levels that impact aphid population increase, with the volatiles produced from crushed leaves having a much larger effect. The C6 aldehydes and alcohols may be components of the fecundity reduction seen with tomato volatiles; however, volatile terpenes showed no effect. These results can be of significance for the genetic alteration of plants for improved aphid resistance.

Key Words—Aldehydes, alcohols, fatty acids, lipids, lipoygenase, pest defense.

*To whom correspondence should be addressed.

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