



## Herbivory Effects on the Chemical Constituents of *Bromus pictus*

Laura Sara Malec and Alicia Beatriz Pomilio\*

*PROPLAME-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, C1428EHA Buenos Aires, Argentine*

\* Research Member of the National Research Council of Argentina (CONICET, Argentine).

### Abstract

Southwestern Patagonian arid rangelands of Argentina are subjected to climate and overgrazing degradation. These selected closings are rich in *Bromus pictus* which is one important grass due to its fodder properties. Therefore, their chemical constituents, chemical composition and occurrence of the main families of chemical compounds were studied in order to establish this grass as a degradation marker in overgrazed Patagonian rangelands.

**Keywords:** *Bromus pictus*; chemical constituents

(Received december 2002, accepted april 2003)



*Bromus pictus* belongs to the family Poaceae, which is the largest family of the monocots. Plants from a selected paddock of the arid patagonian overgrazed rangelands (SW of the Province of Chubut, Argentina) were selected for this research. Both *Bromus pictus* and *Poa ligularis* are grasses of value as damage markers owing to their fodder properties (Soriano, 1983, 1992; Soriano and Brun, 1973; Soriano and Movia, 1986). The lack of chemical information on the former species prompted us to carry out a chemical screening of the main families of compounds occurring in *Bromus pictus*, the chemical analysis of its constituents under overgrazing conditions as well as to determine its chemical composition according to the methods of the AOAC (1990).

## RESULTS AND DISCUSSION

The indicative value of deterioration of a series of structural features of the community was analysed on the basis of the constituents of *Bromus pictus* in some paddocks near to each other in the arid steppe of the southwestern Chubut, Argentina. The floristic diversity (Gottlieb *et al.*, 2001) was smaller in those places that have been subjected to a higher grazing intensity. It has been earlier demonstrated (Soriano, 1992) that the indirect classification of the places was associated to a major sheep load and density of dungs, and also with the fullness of peri-bush grass rings. The position in the classification of these places was positively correlated with the covering of *Poa ligularis* ( $r = 0.90$ ), that of *Bromus pictus* ( $r = 0.67$ ), the sum of the mesophitic grasses ( $r = 0.81$ ) and the shrubs ( $r = 0.78$ ). The most frequent gramineous was *Poa ligularis*. An interaction between the microplace and the history of its grazing management was found in the Patagonian region.

**A chemical screening of *Bromus pictus* was first performed in order to know the occurrence of some families of chemical constituents:**

**I) Alkaloids:** The tests of alkaloids carried out at pH = 3 led to:

1. Mayer's reagent: **negative**.
2. Dragendoff's reagent: **negative**.

### II) Flavonoids:

When carrying out the Shinoda test on the aqueous layer coming from pH:3, a yellowish solution was observed, thus indicating a **negative** result.

The residue was dissolved in ethanol and distributed in 5 test tubes, which were used for the following assays:

**1. Flavonoid glycosides:** The Shinoda test at room temperature led to a transparent solution, indicating a **negative** result.

**2. Leucoanthocyanidins:** In an acid medium (addition of ClH), a pale yellow colour solution was obtained, characteristic of a **negative** result.

**3. Triterpenic steroidal glycosides:** The Liebermann-Burchard test gave a yellowish solution, indicating a **negative** result.

**4. Cardiotonic Glycosides:** The Kedde test gave a slightly yellowish solution, indicating a **negative** result.

**5. Alkaloids:** The residue was dissolved in HONa and then Mayer's reagent was used. **Negative** result.

**Direct assays** were performed on samples of plant material in order to determine the occurrence of **cyanogenic glycosides, alkaloids and saponins**.

### a. Cyanogenic Glycosides:

i. The reaction with a 5% ferrous sulfate solution and 10% hydrochloric acid gave a **negative** result: yellow-coloured paper.

ii. The reaction with a 1% solution of picric acid in ethanol and a 10% solution of sodium carbonate gave after 24 h in the darkness a yellow colour indicating a **negative** result.

**b. Alkaloids:** An acid extraction was performed: The suspension of plant material and 1% hydrochloric acid was heated in a water bath for one hour at 60°C. After cooling down, was filtered, and the occurrence of alkaloids was tested with the following reagents:

- i. Dragendorff: **positive** result.
- ii. Picric acid: **positive** result.
- iii. Silicotungstic acid: **positive** result.
- iv. Mayer: **positive** result.

**c. Saponins:** height of the foam: 1 mm.

Chemical constituents of the *n*-hexane percolate:

The constituents of the *n*-hexane percolate were analysed and as expected non-polar compounds were identified: *n*-hexacosanol and its homologous series, (hepta -, octa -, nona-, deca -, etc); 13-tetradecenal, 9-octadecenal, 1-hexacosanal and its homologous series; pentatriacontane; hopenone; isomultifluorenone; tetradecanoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; estran-3-one; hexadecanoic acid; (*R*)-(-)-14-methyl-8-hexadecin-1-ol; pentadecanal.

### Components identified in the polar extract:

Sugars: HPLC with a selective column for sugars (PbNO<sub>3</sub>) was performed. The following sugars were identified: sacharose, fructose, stachiose, raffinose. Reducing sugars, fructans. Mixtures of beta-glucan 1,3 and 1,4. Absence of starch.

Acids: Non -proteinogenic acids: in the fractions of the soluble nitrogenous compounds there were amino acids that usually are not part of the proteins. The analysis of the non-proteinogenic amino acids led to: the occurrence of



*alpha*-aminoadipic acid as well as absence of acetylornitine and

of pipercolinic acid. Shikimic acid, citric acid and oxalic acid.

Free organic acids: Derivative acids of the cinnamic acid, *trans*-cinnamic acid, caffeic acid, ferulic acid, *p*-coumaric acid, gallic acid, vanillic acid, *p*-hydroxybenzaldehyde, stearic acid (18:0), and unsaturated and hydroxylated derivatives.

In summary, the following results were obtained concerning the constituents of *Bromus pictus*:

#### Screening:

Direct alkaloids	+
Flavoglycones	+
Saponins	+
Pentacyclic triterpenoids	+
Steroids	+

**Characterised:** Lineal alcohols, normal aldehydes of long chain, lineal acids, unsaturated alcohols and with ramifications with methyls (homologous series),

pentacyclic triterpenones (triterpenic ketones), pentacyclic triterpenic alcohols, *alpha,beta*-unsaturated steroidal ketones. Saturated and unsaturated lineal organic acids with the presence of polar functional groups; aromatic acids; Substituted aromatic organic acids, substituted and free simple phenols.

#### Chemical composition of *Bromus pictus*:

There was also necessary to know the quantity of fibers, proteins, sugars, ash, and so on according to AOAC in order to establish the nutritional quality of this grass as well as the lignicity ratio (hardness extent) (Gottlieb *et al.*, 2001).

The chemical composition of *Bromus pictus* is shown in Table 1. The most noteworthy are the high values for fiber and ash. The latter is mostly acid-insoluble, indicating a high silicic acid content. Hydrolysable carbohydrates were also major constituents, but sugar concentration was practically negligible. Protein and lipid proportions were rather low.

**Table 1: Chemical Composition of *Bromus pictus* (g.kg<sup>-1</sup>)**

Moisture	97.8
Ash	258
Ash insoluble in acid	223
Crude protein (N x 6.25)	42.6
Reducing sugars (as glucose)	1.9
Nonreducing sugars (as sacarose)	traces
Hydrolysable carbohydrates (as starch)	186
Crude fiber	294
Crude fat	17.8

On the other hand, *Bromus pictus* is a very good marker of deterioration due to its fodder characteristics. The herbivores consume selectively the individuals of this species together with *Poa ligularis*, causing the decrease of their abundance and even the local extinction of the populations in those places where there is overgrazing. The value of *B. pictus* is as a fodder grass as much as an indicator of deterioration, thus emphasizing how important is to know the chemical structures of its constituents in order why this species is preferred by the herbivores.

## EXPERIMENTAL

### GENERAL.

Infrared absorption spectra (IR) were registered in an IR spectrophotometer with Fourier transform (FT-IR), Mattson, Galaxy 3020 and processed with the WinFIRST

(Mattson) software. The determinations were made in nujol suspensions or in potassium bromide pellets.

The ultraviolet absorption spectra (UV) were determined in solutions of methanol in a Hewlett Packard 8451-A spectrophotometer with diode array.

Shift reagents were used when recording the UV spectra of the flavonoids, as follows: sodium metoxide (NaMeO), 2,5 g of sodium in 100 ml of methanol; 5% aluminum chloride (AlCl<sub>3</sub>) in methanol; aqueous hydrochloric acid (HCl), 1:1; solid sodium acetate (NaAcO) and solid boric acid (H<sub>3</sub>BO<sub>3</sub>).

The spectra of protonic magnetic resonance (1H-RMN) were recorded at 200,1 MHz and those of 13C at 50,3 MHz, in a spectrometer Bruker ACE-200. The deuterated solvent used is indicated in each case; tetrametilsilane (TMS) was used as internal standard.



Mass spectra (MS) were carried out at 70 eV in a Trio-2 VG Masslab spectrometer and processed with the Masslynx (VG) program. Gas-liquid chromatography combined with mass spectrometry (GC-MS) was carried out in a Trio-2

VG coupled to a Hewlett Packard 5890 GC, using a SPB-1 capillary column of 30 m of length x 0,20 mm of internal diameter (i.d.), of fused silica, 1 min at 60°C, 60-290°C (rate: 10 °C/min) and 5 min at 290 °C.

Reversed-phase high performance liquid chromatography (RP-HPLC) was carried out in an LKB Bromma 2249 gradients pump, Rheodyne injection valve, UV-visible LKB-VWM 2141 variable wavelength detector, LKB Bromma 2221 integrator and Bondapak C18 column of 120 mm length x 4,6 mm i.d. x 3 microm of particle diameter (dp).

All solvents were purified prior to distillation. The extracts and/or chromatographic fractions containing organic solvents were concentrated in vacuo at less than 40°C. Aqueous extracts were subjected to lypophilization.

All mixtures are expressed in volume relationships (v/v).

#### CHROMATOGRAPHIC METHODS:

1) **Thin layer chromatography (TLC):** Upward technique was used. Silicagel F254 commercial plates (Merck), 250 microns thickness as well as silica gel G, Cellulose F and Alumina F 254 chromatoplates (Merck) were used. Chromogenic reagents for spot development: sulfuric acid in acetic acid (1:1); 2% FeCl<sub>3</sub> in ethanol; 5% anisaldehyde in isopropanol and Dragendorff reagent according to Thies; Reuther, modification of Vágufalvi. The eluent systems for the chromatography of alkaloids in cellulose TLC (v/v) are: a) Chloroform: cyclohexane: diethylamine (5:4:1); b) Ethyl acetate: pyridine: water (2:1:1); c) n-butanol: acetic acid:water (4:1:2); d) n-butanol: 96% ethanol: ammonium hydroxide (40:20:2). The eluent systems for the chromatography of alkaloids in alumina TLC (v/v): a) Chloroform: diethylamine (9:1); b) Cyclohexane: chloroform: diethylamine (5:4:1); c) Chloroform: acetone: diethylamine (5:4:1); d) Benzene: chloroform (94:6); and e) Benzene: chloroform: diethylamine (5:4:1); f) Chloroform: methanol: 20% ammonium hydroxide (60:40:1); g) Chloroform: methanol saturated with ammonia (9:1).

2) **Column chromatography:** The following sorbents were used for column chromatography: silica gel H (Merck); neutral alumina (Woelm); Sephadex LH-20 (Sigma); polyamide for column (Woelm) and exchange resin Amberlite IRA-400 (HO<sup>-</sup>) (Sigma). Medium liquid chromatography was carried out in silica gel Lobar 60 columns, B and C sizes (Merck) and with silica gel H; the development solvent was impelled by to Prominent-Electronic 1001 pump with an SCJ pulse amortiguator or

with compressed air. The development solvent is indicated in each case.

3) **Gas-liquid chromatography (GLC):** A Hewlett-Packard 5890 equipment with a flame ionization (FID) detector was used. Temperature conditions and the columns used are indicated in each case.

#### GENERAL REACTIONS:

1) **Acetylation:** All acetylations were performed using acetic anhydride and pyridine (1:0.9) at room temperature. The reaction mixture was kept in the dark for 12 - 24 hs and the compounds were isolated after evaporation of the solvents.

2) **Trimethylsilylation:** The sample was dissolved in pyridine and equal quantities of trimethylchlorosilane and hexamethyldisilazane were added. The mixture was stirred and after 5 min in rest was evaporated to dryness. The dried residue was taken with anhydrous chloroform, remaining salts in suspension. In case of using the trimethylsilylated sample to record <sup>1</sup>H-RMN spectra, salts were filtered through a filtering board (Placa filtrante); the filtrate was evaporated and further dissolved in an appropriate quantity of deuterated chloroform. If the compound or mixture of trimethylsilylated compounds was afterwards used for a GLC analysis, the mixture was centrifuged and the supernatant was injected in the chromatograph.

#### Plant material:

Inside the neighboring area to the EEA Inta Río Mayo (Province of Chubut) and on the base of air pictures, and known grazing histories, 4 places were selected in paddocks belonging to the experimental station Inta Río Mayo and 4 in neighboring establishments. Those places were selected that presented in the air pictures marked differences in their texture or shade, contrasts of having wired or of which a grazing history were known. Also 3 closings of 3, 23 and 43 years of antiquity were sampled, that is to say 11 paddocks in total. The objective pursued with this design was to embrace a wide range of heterogeneity in the vegetation presumably generated by the grazing.

#### Extraction of the constituents of the *n*-hexane extract:

Approximately 100 g of dry and milled plant were extracted with *n*-hexane in a Soxhlet. The solvent of the extract was evaporated, and the residue was suspended with silica gel 60 H, using methylene chloride for the suspension. Then a percolation was made through a fritted glass funnel, eluting successively with the following solvents: 1) *n*-hexane (100%); 2) methylene chloride (100%); 3) methylene chloride: ethyl acetate (50: 50); 4) methanol (100%).

The percolates and subfractions obtained were analysed first by TLC with silica gel 60 F2 plates. Gradients of *n*-hexane-ethyl acetate were used as eluents and H<sub>2</sub>SO<sub>4</sub>-



AcOH as spot developer. The fractions were analysed by chemical methods and spectral data. In the case of components present in traces GC-MS was used.

### Chemical screening of the families of compounds occurring in *Bromus pictus*

The plant material was suspended in methanol and the suspension was kept in rest during the night. Then was heated under reflux for 4 hours. After that time, it was filtered warm and the residue was washed with methanol. The filtrate was allowed to cool down, observing any modification, and an aliquot (1 ml) was taken in order to assay **tannins**.

The remaining liquid was evaporated to dryness in vacuo and the residue was taken with 1% HCl (v/v), being afterwards filtered through a Celite layer. The residue of the rounded flask was further treated with 1% HCl and then filtered. The filtrates were combined, and the insoluble residues of the flask with the insolubles retained in the funnel.

Whenever it was difficult to dissolve the residue in HCl, a mild heating was performed in a bath at 50-60 °C, mixing the suspension under magnetic stirring. This treatment produces a residue mixed with celite (**Insoluble A**) and an acid solution (**Filtrate B**).

The **Insoluble A** was put in a test glass, to which chloroform was added and was stirred strongly.

It was observed whether the residue of vegetable origin was dissolved. Without prior filtering the insoluble was added to the suspension as well as anhydrous sodium sulfate in small portions, stirring after each addition. It was kept in rest during one night and then 1 ml of the clear chloroformic solution was taken in order to assay **phytosterols**.

**Steroids** (Liebermann-Burchard's test): Acetic anhydride (1 ml) and concentrated sulfuric acid (5 drops) were added to the chloroformic solution (1 ml) in a tube. When the reaction is positive, after few seconds an intense green colour appears.

**Spot test** (with a spot plate): Acetic anhydride (3 drops) and sulfuric acid (one drop) were added to 3 drops of the chloroformic solution and were well mixed with a thin bar. The colour that indicates a positive reaction appears in few seconds. In the touch badge sometimes the colour is brown-reddish in the center of the mixture and green in the borders.

The acid filtrate B was cooled at room temperature, further basified with concentrated ammonium hydroxide at pH 10, preventing a rise of temperature, and it was further extracted with chloroform (2 x 15 ml) in a separatory funnel, being the inferior chloroformic layer in a dried Erlenmeyer.

When emulsions are formed, the separation of the layers was carried out by centrifugation.

All chloroformic layers were combined to give a chloroformic **extract C**, which was washed once with water. This water and the upper aqueous layer from the chloroformic extraction were combined to conform an **aqueous solution D**.

The chloroformic **extract C** was dried over anhydrous sodium sulfate for a night, then was filtered and the filtrate

was evaporated to dryness in vacuo, being obtained a **residue C**, on which Liebermann-Burchard's test (**steroids**) and the test of **alkaloids** were performed as follows:

**Steroids:** A small portion of the residue C was taken with a glass bar and further dissolved with 3 drops of chloroform. Acetic anhydride (3 drops) and concentrated sulfuric acid (a drop) were added (spot test). An orange colour indicates a positive test. The original chloroformic solution is yellowish and may be used as a control, scoring if this colour is intensified.

**Alkaloids:** The rest of the **residue C** was dissolved in 1% hydrochloric acid (14 ml).

Mild heating in a bath and further stirring with a bar helped dissolution. If total dissolution is not achieved, the insoluble is separated out by decantation or by filtration. To five fractions of 0.2 ml each 1 drop of the following reagent was added: a) Mayer, b) Dragendorff, c) picric acid, d) silicotungstic acid; (see preparation of reagents and evaluation of the test of alkaloids). A precipitate or turbidity in the tube indicates a positive reaction. The final observation is made after 10-15 minutes rest. The test of Dragendorff can be made as a spot test, on paper. The alkaloids can also be analysed in a spot plate, using five drops of the aqueous acid solution and then a drop of each reagent.

To investigate the alkaloids by chromatography, the rest of the solution (0.4 ml) was diluted to 3 ml, basified with concentrated hydroxide ammonium and was extracted with chloroform (3 x 5 ml) in a separatory funnel. The chloroformic extracts were gathered and further dried over anhydrous sodium sulfate and the chloroformic filtrate was evaporated to dryness. The residue was used in order to perform thin layer chromatography.

Some ml from the aqueous solution D (approx. 0.25 ml) were taken to pH 2.5-3.0 (red Congo) with 10% hydrochloric acid (v v), and the **test of alkaloids** with reagent of Mayer was repeated. With a drop in a filter paper was also assayed the test of Dragendorff.

To the remaining **aqueous solution D**, anhydrous sodium sulfate or 220 mg of the decahydrate was added, for milliliter, and was further extracted with a mixture of chloroform-ethanol (3:2) (2 x 15 ml) in a separatory funnel. The organic layers (lower layers) were combined



giving an **extract E**, which washed once with 5 ml of the semi-saturated solution of sodium sulfate, combining the wash liquid with the remaining upper aqueous layer of the extraction chloroform-ethanol. An **aqueous solution F** was obtained. One ml of this last solution was used in order to test **flavonoids**.

**Flavonoids (Shinoda test):** Concentrated hydrochloric acid (8-9 drops) and some magnesium filings (it is advisable the one used for the reaction of Grignard) were added. After 10-15 minutes at room temperature the colour of the solution was observed. A red coloration appears

when the reaction is positive. Isoamyl alcohol (0.5 ml) was added and stirred, and the coloration should pass to the alcohol.

The **chloroformic-ethanolic extract E** was dried for a night over anhydrous sodium sulfate and was evaporated to dryness in vacuo (**Residue E**). The **residue E** was dissolved in ethanol and 0.20 ml of this extract was distributed in five Pyrex tubes, respectively. The content of three of these tubes was taken to dryness with nitrogen. On each of these tubes the following tests were performed:

**1. Flavonoid Glycosides:** Concentrated hydrochloric acid (2-3 drops) was added to the original solution (0.2 ml), as above indicated. Isoamyl alcohol is not used.

**2. Leucoanthocyanidines:** Concentrated hydrochloric acid (2-3 drops) was added to the original solution (0.2 ml) and then heated until boiling. A reddish colour indicated a positive reaction.

**3. Triterpenic steroidal glycosides:** These compounds are assayed in the dry residue by the Liebermann-Burchard's test, just as indicated above. An orange colour indicates a positive reaction.

**4. Cardiotonic Glycosides:** These compounds are assayed in the residue by the reaction of Kedde. The residue was dissolved in 50% ethanol (0.2 ml), and a solution (0.1 ml) of 3,5-dinitrobenzoic acid [1 g of 3,5-dinitrobenzoic acid was dissolved in 100 ml of a 0.5 N solution of potassium hydroxide in aqueous methanol (50%, v/v)] was added to the prior solution. A stable purple colour indicates a positive reaction.

A blank assay is recommended because the colour of the reaction can be modified by the original of the solution. The reaction can also be made with a drop of the solution to be assayed (50% ethanol) on a filter paper, dried off and further treated with the Kedde's reagent. This reaction can be confirmed by the Raymond's reaction, adding a 1% solution (0.1 ml) of *m*-dinitrobenzene in absolute ethanol to the solution (0.2 ml) to be assayed, cooling in an ice-bath and adding a 20% solution (2-3 drops) of sodium hydroxide. An indigo-violet colour that disappeared quickly indicated a positive reaction.

**5. Alkaloids:** The residue was dissolved in 1% hydrochloric acid (0.2 ml) using a bar if it were necessary and a

drop of Mayer's reagent was added to the solution.

**Assay of tannins.** A milliliter of the original methanolic extract was placed in a test tube and taken to dryness under nitrogen. The residue was dissolved in water (0.5 ml) with stirring. To an aliquot (0.2 ml) of this aqueous solution, 1% ferric chloride (1 drop) was added. A dark green, blue or brown colour indicated a positive reaction. The reaction can be made on a filter paper, but the colour is not stable and vanishes. If a spot plate is used, a drop of 1% ferric chloride was added to 3 drops of the solution to be assay. Furthermore, an aliquot (0.2 ml) of 1% jello solution was added to the aqueous solution (0.2 ml). Turbidity or

precipitate indicated a positive reaction. The jello solution was prepared by dissolving jello (1 g; pharmacy quality) in a 10% aqueous solution (100 ml) of sodium chloride.

#### DIRECT ASSAYS

Direct assays are performed on a sample of plant material in order to establish the occurrence of **cyanogenic glycosides, alkaloids and saponins**. These compounds are assayed directly only when the tests of the systematic screening were negative.

**Glicósidos cianogénicos:** a) On the mouth of a 50 ml Erlenmeyer that contains a sample (1 g) of the plant material and 2N sulfuric acid (5 ml), a piece of filter paper is placed moistured with a 10% sodium hydroxide solution and for one minute is further mild heated. On the humid portion of the paper 3 drops of a 5% ferrous sulfate solution are then added, and after two minutes 3 drops of 10% HCl are also added. A blue colour indicated a positive reaction. b) In an Erlenmeyer of 100 ml, original plant material (2 g) covered with water (20 ml) was placed. The Erlenmeyer was covered with a conditioned plug with a hanging paper ribbon absorbed with a 1% solution of picric acid in ethanol and sprayed with a 10% solution of sodium carbonate. The system was left in rest for 24 h in the darkness. An intense pink to dark brown colour indicated a positive reaction.

**Alkaloids:** a) **By acid extraction:** The plant material (0.1-0.5 g) was covered with a volume approx. five times its weight of 1% hydrochloric acid and the suspension was heated in a water bath for one hour at 60°C, stirring sporadically. After cooling down, was filtered, and the occurrence of alkaloids was tested on the filtrate with the specific reagents (*vide supra*).

b) **By alkaline extraction:** Ethyl ether-chloroform-ethanol-ammonium hydroside (density 0.9) (25:8:2.5:1 in volume) the so-called 'Liquor of Prolius' was used for the extraction. A portion of the plant material (0.1-0.5 g) was covered with 5 times the volume of that mixture and was kept standing. After 24 hours, was filtered, the filtrate was evaporated to dryness, and the residue was extracted with 1% hydrochloric acid (1.4 ml), favoring the dissolution of the bases with magnetic stirring. It was further filtered, and the alkaloids were tested on the filtrate.



Reactions were performed with all the specif reagents for alkaloids (*vide supra*).

**Saponins:** A portion (1 g) of the plant material was suspended in cold water (10 ml) in a test tube. After 24 h at room temperature it was heated for 12 minutes in a boiling water bath. It was filtered warm in vacuo (using a Buchner) or through a fluted filter, and washed with cold water until the filtrate had a volume of 10 ml. An aliquot (1 ml) of the filtrate was placed in a Pyrex test tube and strongly stirred for 15 seconds. After 15 minutes of keeping in rest the height of the foam was read.

### Preparation of the reagents and evaluation of the reactions

#### 1) Reagents for testing alkaloids:

**Mayer's reagent:** A solution of mercuric chloride (13,6 g) in water (600 ml), and another of potassium iodide (50 g) in water (100 ml) were prepared. Both solutions were mixed under stirring and the volume was completed to 1000 ml with water.

**Dragendorff's reagent:** Glacial acetic acid (10 ml) was added to a suspension of bismuth subnitrate (0.8 g) in water (40 ml). At the same another solution of potassium iodide (20 g) in water (50 ml) was prepared. Both solutions were mixed together, glacial acetic acid (100 ml) was added and the volume was taken to 1000 ml with water.

**Dragendorff's reagent for cellulose TLC:** Bismuth subnitrate (2.5 g), water (20 ml) and glacial acetic acid (5 ml) were placed in an Erlenmeyer in the order indicated and well mixed together. Then potassium iodide (4 g) dissolved in water (10 ml) was added. Upon mixing an

orange precipitate and an orange solution were obtained. This mother solution must be kept protected from the light (in a dark-glass flask). The spray solution is prepared just prior tu use, diluting 5 volumes of the solution with 10 volumes of glacial acetic acid and taking to 100 volumes with water.

The solution shows good sensibility for almost all bases, except for berberine, yohimbine, trigonelline, theobromine, theophylline, nicotinamide and betaine. In order to detect caffeine it was necessary to use 50 microg of it.

**d) Picric acid:** Picric acid (20 g) was dissolved in warm water (1000 ml). The solution was allowed to cool down and that supernatant was used.

**e) Silicotungstic acid (Bertrand's reagent):** Silicotungstic acid (50 g) ( $\text{SiO}_2 \cdot 12 \text{WO}_3 \cdot x\text{H}_2\text{O}$ ) was dissolved in 6N sulfuric acid (1000 ml).

**f) Chlorplatinic's reagent for cellulose TLC:** This reagent has the advantage of giving varied colours with some alkaloids, which allows to follow its separation and purification steps more easily in the case of mixtures. The reagent was prepared as follows: A solution (45 ml) of 10% potassium iodide, 5% chloroplatinic acid (5 ml) and water (100 ml) were mixed together.

#### 2 Evaluation of the reactions:

**a) Reactions of alkaloids:** Aqueous solutions containing 500, 250 and 100 mg of quinine base in 1% hydrochloric acid (100 ml) were prepared. The reactions were performed taking 0,2 ml of each concentration and adding a drop of each reagent. After 10 min the height of the precipitate was read. Usual results are the following:

Reagent	Conc. 500mg	250 mg	100 mg%
Mayer	2mm	1 mm	turbidity
Dragendorff	5 mm	4 mm	2 mm
Picric acid	3 mm	3 mm	turbidity
Silicotungstic acid	4 mm	3 mm	turbidity
<b>Evaluation</b>	+++	++	+

**b) Reaction of flavonoids:** Ethanolic solutions containing 500, 250 and 100 mg % of rutin were prepared. An aliquot (1 ml) of each was placed in a test tube, 8-9 drops of concentrated hydrochloric acid and some few turnings of Grignard-type magnesium were added to each tube. After keeping at room temperature for 10-15 min, a characteristic red colour then appeared.

**Evaluation.** The following value is given to the colour intensity of each tube:

500 mg : +++ 250 mg : ++ 100 mg: +

**c) Reaction of phytosterols:** Three solutions of cholesterol containing 100, 250 and 500 mg in 100 ml of chloroform were prepared. An aliquot (1 ml) of each concentration was taken to perform the Liebermann-Burchard's test.

#### Evaluation.

100 mg + ; 250 mg ++ ; 500 mg +++.

**d) Reaction of cardiotonic glicósidos (Kedde):** Three solutions of digitoxin (digitonin) containing 20, 50 and 100 mg in water (100 ml) were prepared. An aliquot (0.2



ml) of each concentration was taken to perform Kedde's test.

**Evaluation.** 20 mg +; 50 mg ++; 100 mg +++.

**e) Reaction of saponins:** Three solutions of commercial saponin containing 100, 250 and 500 mg each in water (100 ml) were prepared. The test of foam was performed by stirring.

**Evaluation:** 100 mg, height of the foam 10-12 mm: +  
250 mg, 31-34 mm: ++  
500 mg, 42-25 mm: +++

It can also be performed using digitonin. The results indicated are comparative to each other, but not with those obtained using commercial saponin.

**Evaluation:** 100 mg, height of the foam 10-12 mm: +  
250 mg, 20-25 mg: ++  
500 mg, 30-32 mg: +++

#### Chemical composition of *Bromus pictus*

Chemical composition was analysed according to the method of the AOAC (1990): moisture (100°C, p<100 mmHg), ash (500-550°C), fat (crude) (method 920.39C), total nitrogen (Kjeldahl method, 955.04), reducing and nonreducing sugars (methods 925.05 and 959.11), hydrolysable carbohydrates (methods 920.40A and 959.11), fiber (crude) (method 962.09) and ash insoluble in acid [method 30.008 (AOAC, 1975)].

#### CONCLUSIONS

The big herbivores are exceptional agents as patternmakers of the vegetation (Milchunas *et al.*, 1988). The effects of the herbivores occur in hierarchically structured landscapes (Brown and Allen, 1989). At individual's level, the answer can depend, for example, on the restrictions imposed by the physiology of the plant in order to mobilise reserves or to increase the photosynthetic efficiency of the remanent tissue (Caldwell *et al.*, 1981; Detling and Daintler, 1983; Wallace *et al.*, 1984). At the community level, the studies performed in order to elucidate the modifications induced by grazing demonstrate changes in the floristic composition, in the diversity of the community (Naveh and Whittaker, 1979; Noy-Meir *et al.*, 1989) and in the spatial arrangements of the vegetation (Gibson, 1988).

The changes in diversity associated to grazing have been described as an answer of optimum (Grime, 1977; Connell, 1978), existing a level of load or disturbance intensity that maximizes this variable. Above this level, many species see how their survival capacity is overcome and therefore, the floristic wealth of the community diminishes. On the contrary, at low levels of grazing the species of major fall

competitive aptitude exclude the less capable ones competitively, thus reducing the floristic diversity. In different ecosystems of the world studies have been carried out about the effect of grazing on the structure and the diversity of the vegetable communities (Milchunas and Lauenroth, 1993).

The natural vegetation of the Patagonian region has been subjected for more than 100 years to the grazing for sheep (Soriano, 1983). This has caused a degradation process in several scales of the ecosystem (Soriano y Movia, 1986; Ares *et al.*, 1990) similar to those described for other arid systems of the world (Verstraete, 1986; Dregne, 1991). Therefore, in order to evaluate the magnitude and sense of the changes in the structure of the community induced by grazing, it is fundamental in order to determine which are the processes that determine those changes. On the other hand, to elucidate the extent of association between the structural patterns of the vegetation and use indicators would allow to have a more objective measure of deterioration. At the same time, to understand the processes that take to the pastoral systems to less productive states or on the contrary, those that bear to their recovery, facilitate to develop measures or tools to carry out a more rational use of the natural resources.

The chemical constituents, the families of chemical compounds and the chemical composition of *Bromus pictus* from the area of the EEA INTA Río Mayo and of the surrounding fields (around 350 km<sup>2</sup>).

were studied in order to analyse the extent of association of these data and other variables (León and Facelli, 1981; Golluscio *et al.*, 1982; León and Aguiar, 1985; Golluscio and Sala, 1993).

The homogeneity of the dominant community in the SW region of Chubut, responds to a restriction (the climate) of a high hierarchical level. The grazing would act as an agent of finer grain and therefore of a lower hierarchical level. The characters that are perceived at this level, would be the result of ecological processes of inferior hierarchy (in this case changes that happen in the populational level). This approach to the effects of the grazing (Allen and Hoekstra, 1991), allows to make comparisons with regard to their consequences, on the base of the effect of ecological processes whose scale is defined *a priori*.

The observed results about the diversity of species were in agreement with those obtained for the same environment in other works (Soriano and Brun, 1973; Schlichter *et al.*, 1978). The fact that the grazing reduced the floristic diversity was reported. On the other hand, there would also be an agreement with the general pattern





of the diversity with an increase of grazing intensity in very productive environments and with a short evolutive history of grazing.

### Acknowledgements

Thanks are due to Universidad de Buenos Aires, ANPCYT (Argentina) and CONICET (Argentina) for financial support.

### References

- Allen TFH and TW Hoekstra (1991) Role of heterogeneity in scaling of ecological systems under analysis. En: *Ecological Heterogeneity*, Kolasa J y STA Pickett (eds.). Springer Verlag, New York, pp 47-67.
- Ares J., A.M. Beeskow, M.B. Bertiller, C.M. Rostagno, M.P. Irisarri, J. Anchorena, G.E. Defosé and C.A. Merino. (1990) Structural and dynamic characteristics of overgrazed grasslands of the northern patagonia, Argentina. In: *Managed grasslands: Regional studies*. A. Breymer, (ed.), pp 149-175. Amsterdam: Elsevier.
- Association of Official Analytical Chemists (1975) *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington D.C., 12th edition.
- Association of Official Analytical Chemists (1990) *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington D.C., 15th edition.
- Brown B.J. and T.F.H. Allen (1989) The importance of scale in evaluating herbivory impacts. *OIKOS* **54**: 189-194.
- Caldwell M.M., J.H. Richards, D.A. Johnson, R.S. Nowak and R.S. Dzurec (1981) Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* **50**: 14-24.
- Connell J.H. (1978) Diversity in tropical rain forests and coral reefs. *Science* **199**: 1302-1310.
- Detling J.K. and E.L. Daintler (1983) Defoliation responses of western wheatgrass populations with diverse histories of prairie dog grazing. *Oecologia* **57**: 65- 71.
- Dregne H.E. (1991) Global status of desertification. *Annals of Arid Zone* **30**: 179-185.
- Gibson D.J. (1988) The relationship of sheep grazing and soil heterogeneity to plant spatial pattern in a dune grassland. *Journal of Ecology* **76**: 233-252.
- Golluscio R.A. and O.E. Sala (1993) Plant functional types and ecological strategies in Patagonian forbs. *Journal of Vegetation Science* **4**: 839-846.
- Golluscio R.A., R.J.C. León and S. Perelman (1982) Caracterización fitosociológica de la estepa del oeste del Chubut; su relación con el gradiente ambiental. *Boletín de la Sociedad Argentina de Botánica* **21**: 299-324.
- Gottlieb O.R, Kaplan M.A.C. and Borin MR de MV (2001) *Biodiversidad: Un enfoque integrado entre la Química y la Biología*. Idecefyn.
- Grime J.P. (1977) Evidence for the existence of the three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**: 1169-1194.
- Milchunas D.G., O.E. Sala and W.K. Lauenroth (1988) A generalized model of the effects of grazing by large herbivores on grassland community structure. *The American Naturalist* **132**: 87-106.
- Milchunas D.G. and W.K. Lauenroth (1993) Qualitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* **63**: 327-366.
- Naveh Z. and R.H. Whittaker (1979) Structural and floristic diversity of shrublands and woodlands in northern Israel and other Mediterranean areas. *Vegetatio* **41**: 171-190.
- León R.J.C. and M.R. Aguiar (1985) El deterioro por uso pasturil en estepas herbáceas patagónicas. *Phytocoenologia* **13**: 181-196.
- León R.J.C. and J.M. Facelli (1981) Descripción de una coenoclina en el SW de Chubut. *Rev. de la Facultad de Agronomía* **2**: 163-171.
- Noy-Meir I., M. Gutman and Y. Kaplan (1989) Responses of mediterranean grassland plants to grazing and protection. *Journal of Ecology* **77**: 290-310.
- Schlichter T., R.J.C. León and A. Soriano (1978) Utilización de índices de diversidad en la evaluación de pastizales naturales en el centro-oeste del Chubut. *Ecología* **3**: 125- 132.
- Soriano A. and J. Brun (1973) Valoración de campos en el centro-oeste de la Patagonia: desarrollo de una escala de puntaje. *Revista de Investigaciones Agropecuarias*, INTA, Argentina. Serie 2, Biología y Producción Vegetal, **Vol. X**, N°5.
- Soriano A. (1983) Deserts and semi-deserts of Patagonia. In: *Temperate Deserts and Semi-Deserts*. N.E. West (ed.). Elsevier Scientific Publishing Company, Amsterdam.
- Soriano A. and C.P. Movia. (1986) Erosión y Desertización en la Patagonia. *Interciencia* **11**: 77-83.
- Soriano A. (1992) Present conditions in the Patagonian rangelands, in relation to potential impacts of a global climate change. *Proceedings of a Workshop on Assessing technologies & management systems for Agriculture & Forestry in relation to Global Climate Change*. Canberra, Australia. pp 104-116.
- Verstraete M.M. (1986) Defining Desertification: A Review. *Climatic Change* **9**: 5-18.
- Wallace L.L., S.J. McNaughton and M.B. Coughenour. (1984) Compensatory photosynthetic responses of three african graminoids to different fertilization, watering, and clipping regimes. *Botanical Gazette* **145**: 151- 156.



vol 1 July-September 2003, 30- 38

**Molecular Medicinal Chemistry**

<http://www.idecefyn.com.ar>

**IDECEFYN**

ISSN 1666-888X

39