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Promising features of the legume species *Bituminaria bituminosa* as a novel forage plant and for pharmaceutical purposes

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Introduction An interest in perennial pasture species is arising in Mediterranean rangelands because they may extend and regularise the grazing season relative to annuals. *Bituminaria bituminosa* (L.) Stirt. is a perennial wild legume widespread in the Mediterranean basin. It is generally considered of low palatability because of the oil smell emitted by the aerial part, but ecotypes of good palatability and high browse intake are known. The foliage of this species may contain high concentration of furanocoumarins, which could be harmful to animals (for contact photodermatitis) in addition to possibly limiting forage uptake. On the other hand, there is an increasing demand for furanocoumarins in cosmetics and for the photochemotherapy of some skin diseases. Phenolic compounds may also have health-promoting effects due to their antioxidant properties. Agronomically, *B. bituminosa* is a novel species to Italy. This study aimed at assessing the variation existing in the native Italian germplasm for feeding value and biochemical composition.

Materials and methods Seventeen populations of *B. bituminosa* were collected along the western coast of Italy, including Sicily and some minor islands. The latitude of collection ranged between 36°51' N and 44°02' N, the altitude between 30 m and 820 m a.s.l., the soil pH between 6.3 and 8.3, although 15 sites had pH > 7.0. Plant samples of nine populations were dried at 60°C and ground for determining the crude protein according to the Dumas method, and the neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) by the Goering and van Soest method. The phenolic content of plant extracts was determined by colorimetric method (Folin-Ciocalteu reagent). Furanocoumarin glycosides were hydrolysed enzymatically so far on eight populations, and the free psoralen and angelicin thus obtained were quantified by gas chromatography (GC). The volatile fraction was isolated by steam distillation from fresh aerial tissues of 11 populations and analysed by GC and GC-mass spectroscopy.

Results and discussion The feeding value of leaf biomass was rather good, with mean crude protein exceeding 22% and with acceptable fibre content (Table 1). For comparison, leaf protein in lucerne (high-quality legume species) is around 27-28%, leaf NDF is around 21% and leaf ADF is around 16-18%. The stem quality of *B. bituminosa* was much lower, with protein and fibre contents comparable to those of a mature grass species (Table 1). It has been reported that only about 15% of the aerial biomass in *B. bituminosa* is formed by gross stems while leaves and thin stems form the rest. For the sake of species knowledge, protein and fibre contents were also determined on flowers (which are large and abundant at blossom) and roots (Table 1). The former had interesting protein concentration and less lignin than leaves. Some native populations were noticeable in the current study for their overall high forage quality.

Table 1 Population mean, minimum and maximum values of crude protein, fibre fractions and total phenolic concentrations in leaves (9 populations evaluated) and of psoralen and angelicin in aerial biomass (8 populations evaluated), and mean values (across populations) of feeding value parameters and total phenolic concentration in different plant organs.

| | Crude protein (%) | NDF (%) | ADF (%) | ADL (%) | Total phenolics (mg/g DM) | Psoralen (mg/g DM) | Angelicin (mg/g DM) |
|---------------|-------------------|---------|---------|---------|---------------------------|--------------------|---------------------|
| Mean | 22.3 | 24.0 | 18.6 | 7.4 | 22.8 | 3.8 | 3.7 |
| Minimum | 19.2 | 21.0 | 16.5 | 5.5 | 18.9 | 2.8 | 2.3 |
| Maximum | 25.2 | 28.3 | 21.1 | 10.5 | 26.3 | 5.4 | 4.4 |
| Plant organ : | | | | | | | |
| Leaves | 22.3 | 24.0 | 18.6 | 7.4 | 22.8 | - | - |
| Stems | 7.1 | 70.8 | 57.8 | 11.9 | 5.8 | - | - |
| Flowers | 18.3 | 42.4 | 31.5 | 6.7 | 14.5 | - | - |
| Roots | 8.8 | 58.1 | 43.7 | 11.4 | 5.4 | - | - |

Total phenolic concentration was high in all populations (Table 1) when compared, for instance, with that of lucerne varieties. Phenolics play a role in cell protection from oxidative stresses, and can thus be related to drought tolerance. The antioxidant activity exerted by phenolics may raise an interest on the species as a source of these compounds.

The species confirmed to be an important source of the two furanocoumarins psoralen and angelicin. Some populations, particularly from Sicily, exceeded 8 mg/g DM of total concentration for these two compounds (Table 1).

The analysis of the volatile fraction yielded over 110 compounds in detectable amount, with alcohols and sesquiterpenes being the major classes of compounds. Sulphurated compounds, which might contribute to the strong smell of the species, were present in leaves and flowers but not in stems, and ranged from less than 2 to over 7 µg/g DM. A variation in the smell intensity is likely to occur among population of *B. bituminosa*.