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To cite this version:
Giorgio Borreani, Pier Peiretti, Ernesto Tabacco. Evolution of yield and quality of sainfoin (Onobrychis viciifolia Scop.) in the spring growth cycle. Agronomie, EDP Sciences, 2003, 23 (3), pp.193-201. 10.1051/agro:2002082. hal-00886171

HAL Id: hal-00886171
https://hal.archives-ouvertes.fr/hal-00886171
Submitted on 1 Jan 2003

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Evolution of yield and quality of sainfoin (**Onobrychis viciifolia** Scop.) in the spring growth cycle

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(Received 26 February 2002; accepted 11 June 2002)

Abstract – Sainfoin is a perennial legume with high dry matter (DM) yield during spring growth. The aim of this work was to study the evolution of forage yield and nutritional characteristics over a three-year period. Samples were collected at progressive morphological stages, from vegetative to seed pod. The mean stage by weight (MSW), yield, DM content, crude protein (CP), NDF, condensed tannin, gross energy (GE), organic matter digestibility (OMD) and the net energy for lactation (NEL) were determined. The forage characteristics were regressed on the growing degree days (GDD) and the MSW. The DM yield increased from 0.5 to over 8 t·ha⁻¹ as the growth stage progressed from vegetative to flowering, while the leaf to stem ratio decreased from 8 to 0.3 and the CP from 280 to 130 g·kg⁻¹ DM. The NDF ranged from 230 to 502 g·kg⁻¹ DM and was highly related to the GDD with a $R^2$ of 0.92. The OMD decreased with increasing GDD without any differences over the years ($R^2 = 0.83$). Unexpectedly, the codified morphological stage was unable to predict the OMD evolution, as the code scale was not sensitive enough to describe structural variations that affect the crop over the bud stage to flowering.

1. INTRODUCTION

The BSE crisis, the ban on the use of meat and bone meal in ruminant feeds and the increased demand for soybean products have led to considerable pressure on livestock farmers to produce home-grown alternative forages, that are high in protein and energy [36]. Sainfoin (**Onobrychis viciifolia** Scop.) is a perennial legume that is well adapted to dry hilly environments in calcareous soils and which is useful for grazing, haymaking and silage conservation [11]. Sainfoin has declined
over the last 40 years throughout Europe, basically because of its low persistence in the stand and low regrowth rate after the first spring cut [13, 18]. The reduction of 70% in the area grown in Italy was due to structural changes of Italian agriculture and the gradual disappearance of livestock farms in hilly areas [28]. However, sainfoin compares favourably with lucerne, as far as forage quality is concerned. Moreover, the low level of condensed tannins of this species (20–30 g·kg⁻¹ DM) prevents animals from bloating [15] and reduces protein degradation during ensiling [1] and in the rumen. As a result, the absorption of protein in the small intestine is increased in comparison with some forage legumes [7]. Furthermore, sainfoin results in a higher daily weight gain, in cattle and young lambs, than other legumes utilised at a comparable stage of growth [13, 24]. In addition, sainfoin is a legume with nitrogen-fixing capabilities, enabling it to produce substantial yields without the necessity of nitrogen fertiliser, making it attractive as a break-crop in arable rotation [21].

Due to its distinctive characteristics, sainfoin could be conserved as silage for high quality farm supplies [14]. Ensiling as a conservation technique for herbage offers advantages over traditional haymaking because it is less weather-dependent and allows one to anticipate the period of harvesting, thus resulting in a more flexible crop management. In this context, in addition to changes in DM yield, it is important to define the ensilability and nutritional characteristics of the crop over a wide range of growth stages in order to determine the optimal growth stage to harvest depending on the nutritional requirement of the livestock.

There are two main types of sainfoin, the normal type, which is capable of producing very persistent stands, and the giant type, which is productive over a very short period of time. Since the giant type of sainfoin mainly produces forage during the early spring, it may be useful for filling the autumn-spring gaps of forage supply in dairy systems, which are based on maize silage as an alternative to forage grasses.

The objective of this study was to evaluate the evolution of the yield and pre-harvesting herbage quality of sainfoin during the first spring growth cycle in relation to the quantified morphological stage and some environmental parameters over a three-year period in the Po Plain in Italy.

2. MATERIALS AND METHODS

2.1. Plant material and environmental conditions

The research was carried out over the 1998–2000 period in the Western Po Valley near Torino (44°50′N, 7°40′E, altitude 232 m a.s.l., annual mean temperature 11.3 °C, and annual average rainfall 751 mm) on recent sandy-loam texture alluvial soil (Typic Udifluvents, [32]) with a pH of 7.6. The sand, silt and clay contents of the soil were 480, 430 and 90 g·kg⁻¹ respectively, at 0- to 30-cm depth. Organic C was 11.5 g·kg⁻¹ and organic N was 1.39 g·kg⁻¹. Stands of the giant type of sainfoin were sown at 80 kg·ha⁻¹ of naked viable seed in the last ten days of August. All plots were fertilised with 100 kg P₂O₅·ha⁻¹ and 200 kg K₂O·ha⁻¹ at ploughing. No irrigation or fertiliser were applied after sowing. The temperature and rainfall data were collected from a weather station that was about 80 m from the experiment site. Herbage samples for yield and quality were collected with a Haldrup forage plot harvester on subplots of 12 m² randomly located in plots of 30 × 30 m² with three replicates cut to a 3–4 cm stubble height. Herbage samples were collected 4 to 5 times at progressive morphological stages from the early vegetative to late seed pod stage over the period from the beginning of April to the end of May.

The morphological stage was evaluated on a sample of about 50 stems clipped at ground level and classified according to the 10-stage classification system developed by Kalu and Fick [17] that has been slightly modified by Borreani et al. [4] for sulla (Hedysarum coronarium L.) in the 0–1 stages, as reported in Table I. The mean stage by weight was used to calculate the stage of development for the sainfoin canopy using the following equation:

\[ MSW = \frac{(S \times D)}{W} \]  

where: S = morphological stage number, 0 to 9; D = dry weight of the shoots in stage S; and W = total dry weight of the shoots in all stages.

The composition of the harvested herbage was also determined on 50 stems, dividing the plants into leaves (leaflets plus petioles), stems, inflorescence and dead tissue. The leaf to stem ratio was calculated on the dried material as follows: (leaflets + petioles)/stems.

2.2. Chemical analysis

The herbage samples were immediately dried in a forced-draft oven to constant weight at 65 °C, air equilibrated, weighed, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass a 1 mm screen, and stored for qualitative analyses.

The dried samples were analysed to determine the total N, according to the Dumas method, using a Nitrogen analyser macro-N (Foss Heraeus Analysensysteme, Hanau, Germany).
Yield and quality evolution of sainfoin

The crude protein (CP) (total N × 6.25), ash by ignition to 550 °C, and neutral detergent fibre (NDF) were determined according to Robertson and Van Soest [27]. The gross fibre was determined according to Weende, gross energy (GE) with an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany), and organic matter digestibility (OMD) according to the two-stage rumen fluid technique [30]. The OMD values were expressed in vivo using the regression equation of Goldman et al. [12]. Net energy, expressed as both the net energy for lactation (NE_L) and milk forage units (milk FU), was calculated by inserting the observed values of OMD and GE into the equations proposed by Andrieu and Demarquilly [2]. Fresh samples of the herbage were refrigerated, freeze-dried and ground to pass a 1 mm screen. Extraction of free condensed tannins (CT) was done on freeze-dried samples according to Terril et al. [29] as modified by Piluzza et al. [25]. The extractable CT determination was performed on a Helios spectrophotometer (Unicam Limited, Cambridge, UK) following the butanol-HCl-Fe^{3+} assay [26]. A standard calibration curve was developed by means of purified CT from quebracho tannin. Purification of crude quebracho tannin was done according to Asquith and Butler [3].

CP and NDF analyses were carried out on the leaf and stem fractions for three sampling times in 2000.

### 2.3. Statistical analysis

The data were analysed over the years and harvest maturity through regression analysis. The data were averaged over the field replicates before statistical analysis. The data were then regressed on the MSW, growing degree days (GDD) with a 5 °C base temperature, the total hours of above-horizon sunlight for the growth (TLIT) computed from 1 January to the sampling date, and age in days from 1 January (JDAY), as independent variables. The accumulated GDD was calculated using the following formula for each year:

\[ \sum [(T_{\max} + T_{\min})/2] - 5 °C \]

where \( T_{\max} \) and \( T_{\min} \) are the daily maximum and minimum temperatures, respectively, in °C; negative values were not included in the summation. The GDD value was summed from the occurrence of a daily mean air temperature above 5 °C for five consecutive days to the sampling date. Linear and quadratic regressions were compared using the Draper and Smith [10] stepwise selection procedure to select the best regression model at the 0.05 probability level. All the determination coefficients (R^2) reported in this paper were adjusted for degrees of freedom. The MANOVA analysis of covariance was used to verify the equivalence of the equations for the years [23]. The best equation was selected using criteria of simplicity, coefficient of determination and root mean square error (RMSE).

### 3. RESULTS AND DISCUSSION

#### 3.1. Weather data

The annual air temperature and annual cumulated rain of the three experimental years from January to June and the 25-year mean values are reported in Table II. The three years were characterised by mean temperatures higher than the 25-year values, especially in February. The dates that followed 5 consecutive days with temperatures higher than 5 °C were 11 February 1998, 1 March 1999 and 27 February 2000. All years were characterised by a droughty early spring.

#### 3.2. Morphological stage evolution

The codified morphological stage of the crop followed a similar trend over the years, as reported in Figure 1. The beginning of spring growth happened in the first week of March, the early bud stage was reached at the end of April, while full flowering happened around 20 May.

The regression equations of Table III show that the yield and quality characteristics were more related to the GDD than to the MSW or TLIT, with higher coefficients of determination and halved RMSE values. Unlike some other forage legumes [5], the utilised codified morphological stage is unable to correctly describe the evolution of the yield and herbage quality of sainfoin. The code scale is not sensitive enough to
describe structural variations and to detect the sudden changes in plant tissue components and quality that happen from stem elongation to the beginning of flowering. This can be due to the codification method, which was set up and widely used in America for lucerne [17]. For these reasons the following results are only discussed in relation to the GDD.

3.3. Yield and DM content

In Figure 2, the yield evolution is reported in relation to the GDD and also in relation to the days of the year, for a better understanding of the harvesting moment. The DM yield increased from 0.5 t·ha⁻¹ in the early vegetative stages to 8 t·ha⁻¹ in the late flowering stage without any significant difference over the years.

The high DM yield during the spring growth has also been observed by other authors who reported values higher than 8 t·ha⁻¹ ([9], [20]). A DM yield higher than 4 t·ha⁻¹ at the bud stage makes sainfoin suitable for growth as a winter crop for silage in intensive plain areas. In these areas, sainfoin can be

Table III. Regression equations of DM yield, leaf to stem ratio, crude protein (g·kg⁻¹ DM), condensed tannin (g·kg⁻¹ DM), NDF (g·kg⁻¹ DM) OM digestibility (g·kg⁻¹ OM), and milk Forage Unit (milk FU) per hectare on JDAY, TLIT, MSW and GDD (n = 26).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation model †</th>
<th>Adjusted R²</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM yield</td>
<td>0.000592 JDAY² − 5.81</td>
<td>0.93</td>
<td>0.51</td>
</tr>
<tr>
<td>DM yield</td>
<td>0.0000351 TLIT² − 4.09</td>
<td>0.93</td>
<td>0.51</td>
</tr>
<tr>
<td>DM yield</td>
<td>0.868 MSW + 0.402</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>DM yield</td>
<td>0.0122 GDD − 1.16</td>
<td>0.92</td>
<td>0.57</td>
</tr>
<tr>
<td>Leaf to stem ratio</td>
<td>872 JDAY⁻¹ − 5.95</td>
<td>0.77</td>
<td>0.29</td>
</tr>
<tr>
<td>Leaf to stem ratio</td>
<td>8325 TLIT⁻¹ − 4.81</td>
<td>0.78</td>
<td>0.28</td>
</tr>
<tr>
<td>Leaf to stem ratio</td>
<td>0.111 MSW² − 1.38 MSW + 4.34</td>
<td>0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>Leaf to stem ratio</td>
<td>666 GDD⁻¹− 0.909</td>
<td>0.89</td>
<td>0.20</td>
</tr>
<tr>
<td>CP</td>
<td>52151 JDAY⁻¹ − 237</td>
<td>0.74</td>
<td>24.7</td>
</tr>
<tr>
<td>CP</td>
<td>495148 TLIT⁻¹ − 167</td>
<td>0.75</td>
<td>24.1</td>
</tr>
<tr>
<td>CP</td>
<td>108 MSW⁻¹ + 128</td>
<td>0.43</td>
<td>36.4</td>
</tr>
<tr>
<td>CP</td>
<td>40182 GDD⁻¹ + 61.6</td>
<td>0.84</td>
<td>19.5</td>
</tr>
<tr>
<td>CT</td>
<td>− 0.0247 GDD + 30.8</td>
<td>0.74</td>
<td>1.9</td>
</tr>
<tr>
<td>NDF</td>
<td>6.90 JDAY − 514</td>
<td>0.90</td>
<td>28.7</td>
</tr>
<tr>
<td>NDF</td>
<td>0.498 TLIT − 363</td>
<td>0.91</td>
<td>27.3</td>
</tr>
<tr>
<td>NDF</td>
<td>35.7 MSW + 225</td>
<td>0.67</td>
<td>53.2</td>
</tr>
<tr>
<td>NDF</td>
<td>− 0.000540 GDD² + 1.05 GDD + 40.0</td>
<td>0.92</td>
<td>26.9</td>
</tr>
<tr>
<td>OMD</td>
<td>− 7.18 JDAY + 1521</td>
<td>0.73</td>
<td>55.7</td>
</tr>
<tr>
<td>OMD</td>
<td>− 0.520 TLIT + 1366</td>
<td>0.74</td>
<td>54.5</td>
</tr>
<tr>
<td>OMD</td>
<td>− 36.0 MSW + 752</td>
<td>0.50</td>
<td>76.5</td>
</tr>
<tr>
<td>OMD</td>
<td>0.000888 GDD² − 1.41 GDD + 1017</td>
<td>0.83</td>
<td>44.1</td>
</tr>
<tr>
<td>Milk FU·ha⁻¹</td>
<td>65.1 JDAY − 5814</td>
<td>0.78</td>
<td>446</td>
</tr>
<tr>
<td>Milk FU·ha⁻¹</td>
<td>4.62 TLIT − 4284</td>
<td>0.76</td>
<td>459</td>
</tr>
<tr>
<td>Milk FU·ha⁻¹</td>
<td>− 41.0 MSW² + 776 MSW + 217</td>
<td>0.87</td>
<td>384</td>
</tr>
<tr>
<td>Milk FU·ha⁻¹</td>
<td>5.57 GDD + 149</td>
<td>0.77</td>
<td>511</td>
</tr>
</tbody>
</table>

JDAY, age in days from 1st January; TLIT, total hours of above-horizon sunlight; MSW, mean stage by weight; GDD, growing degree days (°C). † All coefficients are significantly different from zero (t-test, P < 0.05).
Yield and quality evolution of sainfoin harvested for silage at the beginning of May and can be followed by a profitable maize crop.

The DM content (Fig. 3) showed a similar trend over the three years. It was ca. 180 g·kg⁻¹ in the early vegetative stage, decreased quickly to reach a minimum of 110 g·kg⁻¹ at the bud stage (corresponding to GDD 300) and then increased up to 230 g·kg⁻¹ with advancing stages of development. Low DM content at cutting will have a negative effect on the speed of drying and will prolong the required wilting period.

3.4. Plant tissue components, crude protein and tannin content

The values of the plant tissue components (leaf, stem, inflorescence and dead parts), expressed as the relative contribution of each component to the total plant mass, are reported in Figure 4a as averages over the three years. The leaves declined from 90% at the rosette stage to ca. 15% at flowering, when the stems and the inflorescences reached about 75% of the total mass. In the first part of the growth cycle the plant produces mostly leaves (Fig. 4b). After the beginning of stem growth the biomass increase was due only to the development of stems and reproductive organs while the amount of living leaves remained practically constant because of the rapid senescence of the basal leaves. For this reason the leaf to stem ratio decreased from 8.0, at the vegetative stage, to values lower than 0.5 at the early bud stage (Fig. 5). The minimum value of 0.3 observed at flowering was comparable with that observed in the same environment for lucerne at a similar growth stage [6]. The leaf to stem ratio evolution was highly correlated to the GDD following a hyperbolic trend, with a coefficient of determination of 0.89 (Tab. III).
The CP content followed a trend similar to that of the leaf to stem ratio (Fig. 6). The CP decreased quickly from 280 to 130 g·kg$^{-1}$ DM from the early vegetative to early bud stage and stayed constant over the remaining part of the growth cycle, as also observed by Upfold and Wright [31]. This rapid decline was due to the increase in the stem mass, which is characterised by a CP content lower than the leaves, as reported in Table IV. Moreover, the CP content of the leaves stayed almost constant with advancing stages of maturity, while the CP content of the stems slightly decreased from 89 to 80 g·kg$^{-1}$ DM, as observed for all forage legumes.

The condensed tannin content decreased from 27 to 16 g·kg$^{-1}$ DM over the whole growth cycle and was inversely related with GDD, as reported in Table III. Similar levels of CT were found in sulla (*Hedysarum coronarium*) in a Mediterranean environment [25]. These moderate concentrations of CT are known to enhance the forage nutritive value by reducing the protein degradation from the rumen bacteria without depressing rumen fibre digestion or voluntary intake [35].

### 3.5. NDF, organic matter digestibility and energy

The NDF increased from 230 at the rosette stage to 502 g·kg$^{-1}$ DM at full flowering (Fig. 7). It was highly correlated to GDD with a $R^2$ of 0.92 (Tab. III). The increase in NDF is mainly due to the contemporary decrease in the leaf to stem ratio and to the increase from 461 to 576 g·kg$^{-1}$ DM (Tab. IV) in the NDF content of the stems. The strong relation between temperature and structural carbohydrates is clear for sainfoin, as observed in many legume and grass forages [8, 34].

The organic matter digestibility decreased with increasing GDD following a quadratic trend (Fig. 8a), without any differences over the years and with a $R^2$ for all the data of 0.83 (cf. Tab. III). At the late bud stage (MSW = 4), the OMD ranged from 770 to 491 g·kg$^{-1}$ OM for the same code number (Fig. 8b). After the beginning of flowering, the OMD was almost constant and always around 500 g·kg$^{-1}$ OM. These low digestibility values indicated that sainfoin should be harvested before the beginning of flowering for high quality forage, as also suggested for lucerne [16] and for sulla (*Hedysarum coronarium* L.) by Leto et al. [22]. Although sainfoin had a lower OMD than lucerne, animal performance resulted in higher DM intake and higher or equal liveweight gains in many trials [13, 19].

The gross and net energy of pre-harvest sainfoin in relation to GDD are reported in Figure 9. Unlike lucerne and sulla, which are characterised by values of gross energy that are almost constant over the whole spring growth cycle [5], the gross energy of sainfoin showed a slight drop around the early bud stage. However, the mean value of 18.3 MJ·kg$^{-1}$ DM is 

<table>
<thead>
<tr>
<th>Stage</th>
<th>Leaf CP (g·kg$^{-1}$)</th>
<th>Leaf NDF (g·kg$^{-1}$)</th>
<th>Stem CP (g·kg$^{-1}$)</th>
<th>Stem NDF (g·kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late vegetative (24 April)</td>
<td>224</td>
<td>246</td>
<td>89</td>
<td>461</td>
</tr>
<tr>
<td>Late bud (11 May)</td>
<td>225</td>
<td>263</td>
<td>80</td>
<td>546</td>
</tr>
<tr>
<td>Full flowering (19 May)</td>
<td>234</td>
<td>211</td>
<td>81</td>
<td>576</td>
</tr>
</tbody>
</table>
Yield and quality evolution of sainfoin very similar to those observed for other forage legumes and grasses [5, 33].

The NEL declined from 7 to 3.5 MJ·kg⁻¹ DM with advancing maturity, following a similar trend to OMD. When harvested at the bud stage, sainfoin can potentially provide 3000 milk FU per hectare (Fig. 10).

4. CONCLUSIONS

Sainfoin seeded at the end of summer and harvested in the subsequent spring showed good potential to produce forage with a high pre-harvest quality. A DM yield higher than 4 t·ha⁻¹ at the bud stage associated with a NDF content below 400 g·kg⁻¹ DM and digestibility around 650 g·kg⁻¹ OM suggest that sainfoin must be harvested and ensiled from the beginning of May. The data also suggest risks of very low digestibility from later harvests. Even though the CP content of sainfoin was lower than the CP content of lucerne, when compared at the same morphological stage, the presence of moderate condensed tannins can protect protein fractions from degradation during ensiling and in the rumen. This makes sainfoin interesting as an alternative to forage grasses grown during winter and spring. The data suggest that sainfoin can improve the self-sufficiency of dairy farms, in terms of home-grown protein forages.

The codified morphological stage system that was used was unable to predict the changes in the pre-harvesting herbage quality. The method used to describe the morphological stage should therefore be refined for this crop so that it can be used in the field by technicians and farmers.

Acknowledgement: The authors wish to thank Mario Gilardi (Dipartimento di Agronomia, Selvicoltura e Gestione del territorio) for technical assistance in the field and Sara Antoniazzi (Istituto di Scienze delle Produzioni Alimentari – CNR, Torino) for laboratory analysis. This work was funded in part by the Università degli Studi di Torino “Progetto Giovani Ricercatori – 1999”. The work is attributable in equal part to the authors.
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