Grass and Forage Science
The Journal of the British Grassland Society | The Official Journal of the European Grassland Federation

Morphology, tannin concentration and forage value of 15 Swiss accessions of sainfoin (*Onobrychis viciifolia* Scop.) as influenced by harvest time and cultivation site

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Abstract

Fifteen accessions of sainfoin (*Onobrychis viciifolia* Scop.) were characterized for morphological and phenological traits at Reckenholz in the Swiss lowlands (Experiment 1). The effects of accession, harvest time and site on dry-matter yield, condensed tannin (CT) concentration and forage value (Experiment 2) were determined at three sites in Switzerland varying in altitude from 440 to 559 m. Three to four harvests were taken in the first year after establishment (second year of stand) with harvests 1 and 2 chemically analysed. From the characterization in Experiment 1, a clear grouping of single flowering (Communis) and multiple flowering (Bifera) accessions emerged. Additionally, within the Communis accessions, distinct groupings were identified (historical landraces and newly collected ecotypes) based on morphological characteristics. Experiment 2 showed that Communis and Bifera accessions had a similar chemical composition in the first harvest. In the second harvest, Communis accessions were higher in crude protein and CT and lower in neutral and acid detergent fibre concentrations than Bifera accessions. Total dry-matter yields were higher for Bifera accessions. Among the Communis accessions, ecotypes had consistently higher CT concentrations than landraces. *In vitro* organic matter digestibility did not significantly differ among accessions. There were clear effects of harvest time and site for most variables, with clear harvest time × sainfoin group interaction but no site × sainfoin group interactions. The results clearly demonstrate that historical landraces and newly collected ecotypes expand the range of available genetic variation for sainfoin breeding.

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Received 3 September 2010; revised 2 May 2011

Keywords: condensed tannins, digestibility, dry-matter yield, forage value, sainfoin

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial temperate legume, which has been an important component of farming systems in many areas of Europe during the past four centuries. As a legume, its ability to biologically fix atmospheric nitrogen (N) allows successful cultivation without N fertilization (Rochon *et al.*, 2004). Total dry-matter (DM) yields of between 9·5 and 12·5 t ha^{-1} have been reported (Häring *et al.*, 2008; Liu *et al.*, 2008) and are comparable with those from other leguminous forages. Its high protein concentration and palatability along with its non-bloating property make it an interesting alternative to grasses and other legumes. Sainfoin grows well on alkaline soils, and its deep taproot system confers a high level of drought tolerance (Sheldrick *et al.*, 1995). Despite these and several other positive nutritional, animal-health related and environmental attributes (Mueller-Harvey, 2009), there has been a marked decline in sainfoin use under temperate rain-fed conditions in the second half of the 20th century, except for some regions of the Near East. The main reasons for the decline were the advent of cheap inorganic nitrogen fertilizers in the 1950s and the strong focus of legume breeding programmes on lucerne and clovers. However, in Europe and North America, there is currently a revival of interest in forage

doi: 10.1111/j.1365-2494.2011.00811.x

legumes in general (Rochon *et al.*, 2004) and in sainfoin in particular (Piano and Pecetti, 2010). Reasons for the renewed interest include pressures to reduce energy consumption and environmental pollution and to increase biodiversity (Rochon *et al.*, 2004). However, several authors found that longevity of sainfoin may be insufficient under some management practices (Mowrey and Matches, 1991; Peel *et al.*, 2004) indicating a need for genetic improvement and more sound agronomic information (Liu *et al.*, 2008).

Condensed tannins, a very diverse and complex group of phenolic plant secondary metabolites, are thought to be responsible for positive but also negative attributes of legumes containing CT (Mueller-Harvey, 2006). Beneficial effects such as anthelmintic activity may be expected when CT-containing legumes, like sainfoin, are fed to ruminants at optimal dietary concentrations (Aerts *et al.*, 1999; Min *et al.*, 2003), but evidence from recent research findings highlights the need to be very specific when drawing this conclusion (Mueller-Harvey, 2006; Häring *et al.*, 2008). It is known that not only CT concentration but also CT composition can differ within and between plant species (Giner-Chavez *et al.*, 1997; Stewart *et al.*, 2000; Dalzell and Shelton, 2002), and this can result in varying biological properties (Waghorn, 2008).

Investigations on the effects of sainfoin on ruminant performance and health are limited in number and those available are inconsistent in their outcomes. The positive effects of sainfoin on protein metabolism in growing lambs observed by Egan and Ulyatt (1980) could not be confirmed in other studies (Scharenberg *et al.*, 2008, 2009). Also, the anthelmintic property of sainfoin was found only in some studies (Heckendorn *et al.*, 2006; Waghorn, 2008), but not in others (Athanasiadou *et al.*, 2005; Scharenberg *et al.*, 2008). These inconsistencies were repeatedly attributed to variations in CT concentration and composition. Genetic variation among sainfoin cultivars may affect CT concentration and could be one reason for the poor predictability of the beneficial effects of sainfoin, and the choice of a cultivar rich in CT might help to enhance such beneficial effects.

Sainfoin accessions fall into two groups: 'common' or 'single-cut' types, referred to as botanical variety *Onobrychis viciifolia* Scop. var. *communis* Ahlef., and 'giant' or 'multiple-cut' types, botanical variety *Onobrychis viciifolia* Scop. var. *bifera* Hort. (Piano and Pecetti, 2010). In this study, the two groups are referred to by their botanical variety names, 'Communis' and 'Bifera'. Communis types flower in the first growth in spring of each harvest year only, whereas Bifera types allow for at least two flowering cuts per year.

The number of cultivars of sainfoin available is very limited. For example, the current EU catalogue records just 19 cultivars of sainfoin, compared with 350 cultivars of lucerne, (European Commission, 2008). In Switzerland, currently only two cultivars (Visnovsky and Perly, both of the Bifera type) are recommended for use (Suter *et al.*, 2008). Historical landraces or newly collected ecotypes, including Communis types, might expand the range of available genetic variation for selection and breeding. Other factors that may influence variation in CT properties, known from other leguminous forages, include season, soil type (Tiemann *et al.*, 2010) and plant part (leaves, stems, flowers; Gebrehiwot *et al.*, 2002).

Therefore, the objectives of this study were (i) to assess differences in a recently regenerated collection of Swiss accessions, consisting of local landraces, ecotypes and cultivars and (ii) to determine the effects of time of harvest and cultivation site on forage value and CT concentration. A better understanding of the factors influencing forage quality, and their interaction, should assist in developing sound recommendations for sainfoin breeding and utilization.

Material and methods

Experimental sites and sainfoin accessions

Three cultivation sites were chosen for the experiments, which lay on an axis of about 180 km distance. These were Thun (46°45′N, 7°38′E; 559 m a.s.l.) that lies at the edge of the western Swiss lowlands near the northern foothills of the Alps, Ellighausen (47°60′N, 9°14′E; 520 m a.s.l.) and Reckenholz (47°26′N, 8°30′E; 440 m a.s.l.). The latter two are located in the eastern Swiss lowlands. The main soil characteristics and total precipitation, average temperature and relative humidity during the last 30 days prior to the first and second harvests at each of the three sites are given in Table 1.

Fifteen sainfoin accessions were investigated. They included two ecotypes, ten landraces [see Boller and Greene (2010) for definition of ecotypes and landraces] and three commercial cultivars ⁄ breeding lines. The two ecotype accessions (Wiedlisbach and Thun Allmend) originated from permanent, natural grasslands that had never been re-sown and were collected in 2004 (Eric Schweizer, 2006). The landraces (Moiry, Cuarnens, Pompaples, Premier, Sarzens, Vinzel, Echandens, La Rippe, Middes and Perly 66) were retrieved from a collection made by Dr Samuel Badoux (Prangins, Switzerland) in the mid-1950s in western Switzerland where, at that time, on-farm maintenance of sainfoin landraces was still popular (Badoux, 1965). They originated from farms where they had been maintained for decades by re-sowing a new field with sainfoin seed harvested from an old field that had been used for forage production for several years. Seeds of ecotype

Table 1 Soil characteristics and weather data determined during 30 days prior to the respective harvest at each of the three sites.

Site	Thun	Ellighausen	Reckenholz
Soil characteristics			
Soil type	Eutric cambisol	Eutric cambisol	Gleyic cambisol
Texture	Sandy loam	Loam	Clay loam
Organic matter (% org. C)	1·35	1·45	1·65
pH (H_2O)	6·6	6·9	6·8
First harvest			
Harvest date	May 23	May 27	May 29
Total precipitation (mm)	51·0	18·3	49·4
Average temperature (°C)	15·9	13·7	15·0
Relative humidity (%)	73·3	69·8	67·1
Second harvest			
Harvest date	July 9	July 8	July 10
Total precipitation (mm)	104·8	66·7	106·1
Average temperature (°C)	18·4	19·0	15·9
Relative humidity (%)	75·1	74·8	74·4

accessions and landraces were multiplied in spatial isolation as part of a project intended to preserve Swiss genetic resources of sainfoin (Eric Schweizer, 2006). Seeds of the cultivars Visnovsky (maintainer: Oseva, Czech Republic) and Perly (maintainer: Agroscope Reckenholz-Tänikon ART, Switzerland) and of the breeding line OV0505 (ART) were obtained from breeders. The registered cultivar, Perly, and the candidate cultivar, OV0505, both trace back to breeding material selected from a landrace from the village of Perly (Canton Geneva, Switzerland). This landrace was named Perly 66 in the present study. All accessions were later assigned to either Communis or Bifera type based on the characterization reported in Table 2.

Field experiments

The two experiments commenced in spring 2007. Experiment 1 was carried out exclusively at the Reckenholz site for morphological and phenological characterization of the 15 accessions. Sixty individual plants of each accession were raised in a greenhouse for 2 months, trimmed and planted in a field nursery on 10 May 2007 in a complete randomized block design in six replications of ten individuals, planted in rows at 50 cm apart and having a between-plant distance of 30 cm. In Experiment 2, all 15 accessions were established in plots by sowing at a rate of 50 kg ha^{-1} between 3 and 20 April 2007 at all three sites in 9·0-m^2 plots (1·5 × 6·0 m) as a randomized complete block design with three replications. All plots received a single application of P and K fertilizer in November prior to sowing (corresponding to 90 kg P_2O_5 and 275 kg K_2O ha^{-1}) and no nitrogen dressing at any time during the experiments. As no artificial inoculants were applied, nitrogen nutrition of sainfoin relied primarily on symbiosis with naturally occurring Rhizobia. Nodulation was generally good, and there were no signs of nitrogen deficiency, as confirmed by crude protein concentrations of at least 156 g kg^{-1} DM (see Table 4).

Data collection and sample preparation

Observations in Experiment 1 were made in autumn 2007 (growth habit) and in spring 2008 (growth habit, leaf, stem and flowering characteristics). Growth habit and tendency to aftermath flowering were scored on a visual scale with 9 grades, where 1 = very erect/no flowers and 9 = very prostrate/very abundant flowering (Delgado *et al.*, 2008). Leaf characteristics were measured on the leaf supporting the first inflorescence at flowering. The dates of bud and flower appearance were recorded when three stems per plant showed a bud or an open flower. Because stem growth appeared to be indeterminate, instead of measuring the stem length at full development, length of the longest stem was measured up to the node supporting the first inflorescence and up to the tip of the first fully opened inflorescence.

In Experiment 2 (total n = 270), plant material was harvested at a stubble height of about 5 cm in late May (first harvest) and again in early July 2008 after 42 d of regrowth (second harvest). Before harvest, plants in each plot were scored for vigour on a scale from 1 to 9 where 1 = most vigorous and 9 = least vigorous and in the case of the July harvest for aftermath flowering, where 1 = no flowers and 9 = abundant flowering. One to two more harvests were performed between August 2008 and late October 2008 for the determination of total DM yield (DMY). The DMY of each plot was

Table 2 Morphological and phenological characteristics of the sainfoin accessions (Experiment 1).

Group/Accession	GHa	GHs	DBe	DFe	SLgN1	SLgF1	PedL	LL	LtL	LtB	AF1	AF2
Communis ecotype avg.	5·37^{A}*	5·30^{A}	3·89^{D}	18·3^{C}	49·0^{C}	85·2^{C}	36·1^{A}	13·2^{A}	25·7^{AB}	7·98^{AB}	2·01^{B}	1·16^{B}
Wiedlisbach	5·30^{ab}	5·26^{ab}	1·34^{g}	15·2^{e}	41·6^{g}	77·7^{h}	36·1^{abc}	14·5^{a}	25·8^{cd}	7·92^{abc}	1·70^{cd}	1·01
Thun Allmend	5·44^{a}	5·33^{a}	6·44^{ef}	21·4^{cd}	56·4de	92·6^{ef}	36·1^{abc}	12·0^{bc}	25·6^{cd}	8·03^{ab}	2·32^{cd}	1·32
Communis landrace avg.	4·61^{B}	4·35^{B}	5·64^{C}	20·1^{B}	52·1^{C}	88·2^{C}	36·1^{A}	12·8^{A}	27·0^{A}	8·26^{A}	2·21^{B}	1·15^{B}
Moiry	5·21^{ab}	4·82^{abc}	6·67^{def}	21·5^{cd}	58·7de	94·9^{def}	36·3^{ab}	11·8^{bcd}	27·0^{abcd}	8·32^{a}	2·83^{c}	1·33
Cuarnens	5·09^{abc}	4·74^{abcd}	4·79^{f}	18·9^{d}	45·3^{fg}	82·1^{gh}	36·9^{ab}	13·3^{ab}	26·5^{abcd}	7·80^{abc}	1·63^{d}	1·04
Pompaples	4·24^{cde}	4·27^{cdefg}	4·44^{f}	18·9^{d}	51·0^{efg}	87·0^{fg}	36·0^{abc}	12·7^{ab}	25·8^{cd}	8·30^{a}	2·08^{cd}	1·02
Premier	4·51^{bcd}	4·21^{cdefg}	5·60^{ef}	21·0^{cd}	54·2^{ef}	88·9^{efg}	34·8^{abc}	12·5^{b}	28·0^{abc}	8·69^{a}	2·55^{cd}	1·09
Sarzens	4·08de	4·11^{cdefg}	5·91^{ef}	19·6^{d}	51·7^{ef}	87·3^{efg}	35·7^{abc}	13·0^{ab}	25·9^{bcd}	8·16^{ab}	2·40^{cd}	1·45
Vinzel	4·51^{bcd}	3·94^{defg}	6·40^{ef}	20·8^{d}	52·0^{ef}	88·7^{efg}	36·9^{a}	13·3^{ab}	29·0^{ab}	8·32^{a}	1·75^{cd}	1·00
Bifera landrace avg.	3·72^{C}	3·95^{C}	9·61^{B}	25·9^{A}	70·8^{B}	101·8^{B}	31·0^{B}	10·2^{B}	26·7^{AB}	7·56^{B}	6·04^{A}	4·22^{A}
Echandens	3·80de	3·77^{efg}	10·38^{ab}	26·9^{a}	73·4^{bc}	102·1^{bcd}	28·6^{e}	9·5^{ef}	25·4^{cd}	6·73^{c}	6·52^{a}	3·97
La Rippe	3·56^{e}	4·63^{abcde}	9·24^{bcd}	26·0^{ab}	71·8^{bc}	105·9^{bc}	34·1^{abcd}	11·4^{bcde}	29·4^{a}	8·58^{a}	5·23^{b}	3·74
Middes	3·85de	3·63^{fg}	7·85^{cde}	23·7^{bc}	64·5^{cd}	96·5^{cde}	31·9^{bcde}	10·3^{cdef}	26·6^{abcd}	7·88^{abc}	6·14^{ab}	4·66
Perly 66	3·68de	3·76^{efg}	10·96^{ab}	27·0^{a}	73·3^{bc}	102·8^{bcd}	29·5de	9·6^{ef}	25·4^{cd}	7·03^{bc}	6·26^{ab}	4·50
Bifera cultivar avg.	3·83^{C}	3·87^{C}	11·09^{A}	26·9^{A}	81·5^{A}	111·8^{A}	30·2^{B}	9·6^{B}	25·6^{B}	7·47^{B}	5·84^{A}	4·16^{A}
OV0505	3·93de	3·68^{fg}	10·28^{abc}	25·5^{ab}	73·8^{bc}	105·2^{bc}	31·3^{cde}	10·0^{def}	26·2^{abcd}	7·74^{abc}	5·98^{ab}	3·95
Perly	3·24^{f}	3·45^{g}	11·02^{ab}	27·1^{a}	78·8^{b}	108·7^{b}	29·9de	9·7^{ef}	26·8^{abcd}	7·91^{abc}	5·99^{ab}	3·93
Visnovsky	4·34^{cde}	4·48^{bcdef}	11·97^{a}	28·1^{a}	91·9^{a}	121·5^{a}	29·5de	9·1^{f}	23·9^{d}	6·75^{c}	5·54^{ab}	4·59
Min. *n* per accession	43	41	43	42	42	42	42	42	42	42	41	34
Max. *n* per accession	60	58	59	55	55	55	55	55	55	55	58	58
Average SE of accession (group) LS mean	0.18	0.18	0.58	0.66	2.24	2.23	1.04	0.42	0.67	0.27	0.27	0.26
P values												
Group	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	0·006	<0·001	<0·001	<0·001
Accession (group)	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	0·035	<0·001	<0·001	<0·001	<0·001	0·158

GHa, growth habit in autumn of planting year (1 = very erect, 9 = very prostrate); GHs, growth habit in spring (1 = very erect, 9 = very prostrate); DBe, date of bud emergence (days after April 30); DFe, date of flower emergence (days after April 30); SLgN1, length of stem up to 1st flowering node (cm); SLgF1, length of stem including 1st flower (cm); PedL, length of peduncle (cm); LL, length of multifoliate leaf (cm); LtL, Length of third leaflet from tip (mm); LtB, width of third leaflet from tip (mm); AF1, intensity of aftermath flowering after 1st harvest (1 = no flowers, 9 = very abundant flowering); AF2, intensity of aftermath flowering after 2nd harvest (1 = no flowers, 9 = very abundant flowering).

*Within a column, means followed by different superscripts differ significantly ($P < 0·05$). Capital letters indicate significant differences among groups of accessions, and lower case letters indicate significant differences among individual accessions.

Table 3 Dry-matter yield, vigour and aftermath flowering of the sainfoin accessions averaged over 3 sites (Experiment 2).

Group / Accession	DMY harvest 1	DMY harvest 2	TDMY	Mean Vigour	SYF	NHAF
Communis ecotype avg.	6·49^{AB*}	2·59^{B}	9·83^{B}	5·74^{A}	1·76^{B}	39·2^{B}
Wiedlisbach	6·58^{abc}	2·75^{abcd}	10·24^{abc}	5·16^{bcd}	1·61^{f}	38·3^{f}
Thun Allmend	6·40^{abc}	2·42^{cd}	9·41^{c}	6·31^{a}	1·92^{ef}	40·0^{f}
Communis landrace avg.	6·77^{A}	2·67^{B}	10·30^{B}	4·64^{B}	1·61^{B}	42·5^{B}
Moiry	6·58^{abc}	2·31^{d}	9·81^{bc}	5·82^{ab}	1·56^{f}	43·5^{def}
Cuarnens	7·46^{a}	2·80^{abcd}	11·00^{abc}	4·59^{cde}	1·39^{f}	41·9^{f}
Pompaples	6·69^{abc}	2·86^{abcd}	10·46^{abc}	4·27de	1·47^{f}	42·9^{ef}
Premier	6·86^{ab}	2·61^{bcd}	9·90^{bc}	5·47^{abc}	2·21^{e}	39·6^{f}
Sarzens	6·23^{bcd}	2·65^{bcd}	9·95^{abc}	3·60^{e}	1·48^{f}	46·2^{cdef}
Vinzel	6·81^{ab}	2·77^{abcd}	10·71^{abc}	4·07^{e}	1·53^{f}	41·1^{f}
Bifera landrace avg.	6·01^{BC}	3·49^{A}	11·39^{A}	3·51^{C}	6·39^{A}	61·6^{A}
Echandens	5·61^{cd}	3·66^{ab}	11·11^{abc}	3·62^{e}	6·57^{bc}	57·7^{bcd}
La Rippe	5·80^{bcd}	3·52^{abc}	11·64^{ab}	2·39^{f}	6·32^{c}	69·6^{ab}
Middes	6·31^{bc}	3·59^{ab}	11·72^{ab}	3·84^{e}	7·08^{b}	56·9^{bcde}
Perly 66	6·33^{bc}	3·20^{abcd}	11·10^{abc}	4·19de	5·58^{d}	62·3^{b}
Bifera cultivar avg.	5·82^{C}	3·47^{A}	11·66^{A}	3·01^{D}	6·84^{A}	65·4^{A}
OV0505	6·38^{abc}	3·21^{abcd}	11·50^{abc}	3·81^{e}	6·37^{c}	56·8^{bcde}
Perly	5·89^{bcd}	3·43^{abc}	11·43^{abc}	3·64^{e}	6·40^{c}	57·9^{bc}
Visnovsky	5·20^{d}	3·78^{a}	12·04^{a}	1·57^{f}	7·74^{a}	81·7^{a}
n per accession	9	9	9	9	9	3
Average SE of harvest × accession (group) LS mean	0·205	0·208	1·35	0·24	0·12	2·73
P values						
Site	0·013		0·251	0·097	<0·001	–
Harvest	<0·001		–	<0·001	<0·001	–
Group	<0·001		<0·001	<0·001	<0·001	<0·001
Site × group	0·545		0·542	<0·001	<0·001	–
Accession (group)	0·028		<0·001	<0·001	<0·001	<0·001

DMY, dry-matter yield (t ha^{-1}); NHAF, natural height in aftermath (cm); TDMY, total DMY from three harvests at Thun and four harvests at Ellighausen and Reckenholz; mean vigour (visual evaluation scale of 1 = most vigorous and 9 least vigorous); SYF, flowering in seeding year (visual evaluation scale of 1 = no flowers to 9 = very abundant flowering).
*Within a column, means followed by different superscripts differ significantly ($P < 0{\cdot}05$). Capital letters indicate significant differences among groups of accessions, and lower case letters indicate significant differences among individual accessions.

determined gravimetrically at harvest using a plot harvester to determine fresh biomass yield and by taking subsamples for the determination of DM concentration after drying at 105°C for 24 h. For analysis of chemical composition, additional representative samples of fresh biomass were put into plastic bags and stored at −20°C. Subsamples were later lyophilized (Christ Delta 1–24 LSC, Osterode, Germany) and ground to pass a 1-mm screen (Brabender mill, Brabender, Duisburg, Germany).

Laboratory analyses

The nutrient composition of the lyophilized samples was analysed by standard methods. The DM concentration of the lyophilized samples was quantified thermo-gravimetrically by heating at 105°C for 3 h (LECO TGA 601; Mönchengladbach, Germany). Total ash (TA) was determined by dry-ashing at 550°C for 4 h. The N concentration of the samples was analysed using the Kjeldahl method (AOAC, 1995; procedure no. 988·05). Crude protein (CP) was calculated as 6·25 × N. Neutral (NDF) and acid detergent fibre (ADF) were analysed following standard protocols (AOAC, 1995; procedure no. 973·18) using an ANKOM 200 ⁄ 220 Fiber Analyser (Ankom Technology Corporation, Fairport, NY, USA) where NDF was assayed with heat-stable amylase and sodium sulphite. Both NDF and ADF were expressed without residual ash after incineration at 500°C for 1 h. Minerals (Ca, P, Mg, Na and K) and trace

Table 4 Chemical composition and *in vitro* ruminal degradation characteristics of the sainfoin accessions averaged across harvests 1 and 2 (n = 18 per accession, Experiment 2).

Group/Accession	OM	CP	NDF	ADF	CT	IVDOM	GP_n	GP_p	PEG	NEL
Communis ecotype avg.	936^{B}*	174^{A}	382^{B}	351^{B}	67·6^{A}	59·7	45·6	49·0^{A}	7·32^{A}	5·74^{A}
Wiedlisbach	937	174^{abc}	383^{def}	352	68·7^{a}	59·6	45·7	49·9^{a}	8·99^{a}	5·76
Thun Allmend	935	175^{abc}	381^{ef}	350	66·4^{ab}	59·8	45·5	48·2^{ab}	5·65^{b}	5·72
Communis landrace avg.	937^{AB}	175^{A}	388^{B}	352^{B}	61·4^{B}	60·5	45·8	47·7^{B}	4·24^{B}	5·73^{A}
Moiry	936	175^{abc}	393^{bcdef}	356	60·2^{c}	59·6	45·3	47·3^{ab}	4·31^{b}	5·68
Cuarnens	939	169^{bc}	395^{bcdef}	350	58·0^{cd}	61·3	46·3	47·7^{ab}	3·40^{b}	5·75
Pompaples	936	169^{bc}	386^{def}	351	64·2^{abc}	61·2	46·2	48·4^{ab}	4·06^{b}	5·70
Premier	938	182^{a}	383^{def}	349	60·8^{bc}	60·9	45·3	47·3^{b}	4·95^{b}	5·73
Sarzens	937	174^{abc}	393^{cdef}	357	61·8^{bc}	59·8	45·8	47·5^{ab}	4·04^{b}	5·69
Vinzel	938	181^{ab}	378^{f}	349	63·5^{abc}	60·2	46·2	48·1^{ab}	4·66^{b}	5·83
Bifera landrace avg.	939^{A}	164^{B}	408^{A}	363^{A}	53·5^{C}	60·1	45·5	47·5^{B}	4·77^{B}	5·64^{B}
Echandens	939	164^{cd}	414^{ab}	366	50·5^{e}	59·9	45·0	46·8^{b}	5·05^{b}	5·59
La Rippe	938	157^{d}	417^{a}	365	51·7^{e}	59·9	47·0	48·9^{ab}	4·64^{b}	5·75
Middes	938	166^{cd}	408^{abc}	363	53·2de	60·1	44·3	46·5^{b}	4·83^{b}	5·51
Perly 66	939	171^{abc}	394^{bcdef}	356	58·6^{cd}	60·4	45·7	47·8^{ab}	4·57^{b}	5·69
Bifera cultivar avg.	936^{B}	165^{B}	406^{A}	367^{A}	55·0^{C}	60·4	45·5	47·5^{B}	5·21^{B}	5·64^{B}
OV0505	936	171^{bc}	403^{abcd}	364	59·0^{cd}	59·9	44·9	46·8^{b}	4·68^{b}	5·67
Perly	937	170^{bc}	402^{abcde}	363	59·1^{cd}	60·6	45·4	48·1^{ab}	6·42^{b}	5·63
Visnovsky	935	156^{d}	413^{abc}	372	46·9^{e}	60·5	46·1	47·8^{ab}	4·55^{b}	5·64
Average SE of accession (group) LS mean	1·0	2·9	4·6	4·2	1·31	0·50	0·57	0·54	0·76	0·060
P values										
Group	<0·001	<0·001	<0·001	<0·001	<0·001	0·150	0·753	0·007	<0·001	0·033
Accession (group)	0·082	<0·001	0·002	<0·457	<0·001	0·247	0·139	0·015	0·045	0·268

OM, organic matter (g kg^{-1} DM); CP, crude protein (g kg^{-1} DM); NDF, neutral detergent fibre (g kg^{-1} DM); ADF, acid detergent fibre (g kg^{-1} DM); CT, condensed tannins (g kg^{-1} DM); IVDOM, *in vitro* digestibility of organic matter (%); GP_n, gas production without PEG (mL 24 h^{-1}); GP_p, gas production with PEG (mL 24 h^{-1}); PEG, increase in gas production because of PEG (% of GP_n); NEL, net energy lactation (MJ kg^{-1} DM).

*Within a column, means followed by different superscripts differ significantly ($P < 0·05$). Capital letters indicate significant differences among groups of accessions, and lower case letters indicate significant differences among individual accessions.

elements (Cu, Fe, Mn and Zn) were determined using an inductive-coupled plasma optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin-Elmer, Schwerzenbach, Switzerland) after dry-ashing and solubilization in 15·6 M nitric acid. Ether extract (EE) was determined by pressurized solvent extraction (PSE) with petrol-ether (fast PSE; Applied Separations, Allentown, PA, USA) by heating at 125°C with 100 bar for 3 × 12 min.

Concentrations of total CT in the lyophilized plant materials were determined by the method of Terrill *et al.* (1992). For each plant sample, duplicate portions of 500 mg of lyophilized material were used. The absorbance of red anthocyanidin products, characteristic of CT, was read at 550 nm on a UV/VIS Spectrometer (PerkinElmer, Schwerzenbach, Switzerland) with the use of appropriate blanks to account for background absorbance. A CT standard, used for the determination of CT concentration in all 15 accessions, was prepared from the accession Visnovsky (one of the commercial cultivars) based on the method of Stewart *et al.* (2000) as modified by Sivakumaran *et al.* (2004).

In vitro incubations

The *in vitro* digestibility of organic matter (IVDOM) was determined following the two-stage technique developed for the *in vitro* digestion of forage crops as described by Tilley and Terry (1963) with the only modification being that the samples were lyophilized.

To assess *in vitro* ruminal nutrient degradation characteristics and to estimate the energy concentration of the sainfoin accessions, the Hohenheim gas test was used. The test was performed according to the method described by Menke and Steingass (1988) with the following modifications. The 270 sainfoin samples were incubated in a random sequence at approximately 200 mg in 12 successive runs (total n per accession = 18). Each of the 270 sainfoin samples was incubated both without and with polyethyleneglycol (PEG, 8000; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). This compound is known to bind tannins and therefore inhibit their biological effect (Makkar *et al.*, 1995). A PEG solution with unreduced buffer was produced to obtain a minimum ratio of PEG:CT of 1·5:1 (Tiemann *et al.*, 2008). To adjust for variations between and within runs, two incubation units each, containing either standard hay (University of Hohenheim, Institute of Animal Nutrition, Stuttgart, Germany) or no feed, referred to as blanks, were also included. Finally, lyophilized samples (n = 3 per run) of *Trifolium pratense* (<5 g CT kg^{-1} DM), ground to pass a 1-mm screen (Brabender mill no. 880804; Brabender, Duisburg, Germany), were also incubated in each run as a low-tannin legume control. Feed was incubated together with 30 mL of ruminal fluid/buffer mixture (1:2; v:v) for 24 h at 39°C. Ruminal fluid was collected from two rumen-fistulated Brown Swiss cows receiving hay *ad libitum* and supplemented with minerals. After 24 h of incubation, fermentation gas production (GP) was read from the calibrated scale printed on the glass syringes. The GP was corrected by subtracting the GP for blanks.

Calculations and statistical analysis

The total DMY represents the sum of all three to four harvests, whereas all chemical composition data across harvests only include harvests one and two. Net energy lactation (NEL) was calculated by inserting the values of GP, CP and EE into the equation proposed by Menke and Steingass (1988).

Data were analysed using the procedure Mixed Models in SYSTAT (Vs. 13, SYSTAT Software, Inc., Chicago, IL, USA). In Experiment 1, the model included group and accession within group as fixed factors and replicate (actually a blocking factor) and error as random factors:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + b_k + e_{ijk}$$

Y_{ijk}, response in block k for accession j in group i; μ, general mean; α_i, fixed effect of group i; $\beta_{j(i)}$, fixed effect of accession j in group i; b_k, random effect of block k; e_{ijk}, random error.

In Experiment 2, the model included site, harvest, group, harvest × site, harvest × group, site × group, accession within group, site × accession within group and harvest × accession within group as fixed factors and block within site and error as random factors:

$$\begin{aligned} Y_{ijklmn} = {} & \mu + \tau_i + \gamma_j + \alpha_k + (\tau\gamma)_{ij} + (\gamma\alpha)_{jk} \\ & + (\tau\alpha)_{ik} + \beta_{l(k)} + (\tau\beta)_{il(k)} + (\gamma\beta)_{jl(k)} \\ & + b_{m(i)} + e_{ijklmn} \end{aligned}$$

Y_{ijklmn}, nth response in block m of site i for accession l in group k at jth harvest; μ, general mean; α_k, fixed effect of group k; $\beta_{l(k)}$, fixed effect of accession l in group k; γ_j, fixed effect of harvest j; τ_i, fixed effect of site i; $(\tau\gamma)_{ij}$, $(\gamma\alpha)_{jk}$, $(\tau\alpha)_{ik}$, $(\tau\beta)_{il(k)}$, $(\gamma\beta)_{jl(k)}$, interactions of fixed effects; $bm_{(i)}$, random effect of block m at site i; e_{ijklmn} random error.

The statistical parameters were estimated by restricted maximum likelihood (REML) methods for both models.

Multiple comparisons among means were performed with Tukey–Kramer's test. Linear contrasts were used to compare the gas production from the low-tannin legume used as control and the GP from all sainfoin incubations.

Principal component analysis (PCA) was conducted on the correlation matrix of the data set of morphological and phenological characteristics. The Kaiser–Meyer–Olkin (KMO) measure verified the sampling adequacy for the analysis, KMO = 0·61 (Field, 2009). An initial analysis was run to obtain eigenvalues for each component of the data. Two components had eigenvalues over Kaiser's criterion of 1 and in combination explained 86·1% of the variance. The multivariate statistics was run on a SPSS Version 17.0 statistical package (SPSS Inc. Chicago, IL, USA)

Results

Effect of accession

Accessions varied greatly in morphological and phenological characteristics (Table 2). The characteristic 'aftermath flowering' (AF1 and AF2) allowed for a distinct classification of the accessions into eight Communis accessions with very little and seven Bifera accessions with very strong, aftermath flowering. Although aftermath flowering varied significantly within Communis and Bifera accessions, the difference in aftermath flowering score was at least 2·1 units between the weakest flowering Bifera and the strongest flowering Communis type at both scoring dates. The two ecotypes, Wiedlisbach and Thun Allmend, belonged to the Communis type, and all three cultivars (OV0505, Perly and Visnovsky) were of the Bifera type. Both Communis and Bifera types were represented among the landraces.

Though differences for Experiment 1 in growth habit were less pronounced than for aftermath flowering, accessions of both Communis groups (landraces and ecotypes) were more prostrate ($P < 0·05$) than accessions of the two Bifera groups (landraces and cultivars) in both the autumn of the planting year (GHa) and the spring of the following year (GHs). Among all accessions, Wiedlisbach had buds (DBe) and flowers (DFe) emerging earliest ($P < 0{\cdot}05$). The two Communis groups had buds and flowers emerging earlier than the two Bifera groups. Compared with the Bifera accessions, the stems up to the first flowering node (SLgN1) and up to the tip of the first flower (SlgF1) were much shorter ($P < 0{\cdot}05$) in Communis accessions. Furthermore, Communis accessions had longer ($P < 0{\cdot}05$) peduncles (PedL), but shorter natural heights in the aftermath (NHAF) compared with Bifera accessions (Table 3). The cultivar Visnovsky distinguished itself as the accession with latest flowering, longest stems and by far the greatest natural height in the aftermath of flowering. Communis accessions had longer ($P < 0{\cdot}05$) multifoliate leaves (LL) at the standard position (first flowering node) than Bifera accessions, but no clear differences in the length and the width of the leaflet (LtL and LtB) were found between Communis and Bifera accessions.

The extracted components of the principal component analysis carried out on the morphological and phenological data concentrated 86·1% in the first two principal components (74·1% on PC 1 and 12·1% on PC 2). The variables mainly loading on PC 1 were DFe (date of flower emergence), DBe (date of bud emergence) and AF1 (intensity of aftermath flowering after first harvest). For PC 2, the main variables were LtL (length of third leaflet from tip) and LtB (width of third leaflet from tip). On the score plot (Figure 1), which shows the location of the various accessions in the multivariate space of the first two principal component score vectors, Communis accessions were clearly arranged in its two groups (landraces and ecotypes), while the Bifera accessions were more dispersed in space with no clear differentiation between landraces and cultivars.

Communis landraces had higher DMY ($P < 0{\cdot}05$) in the first harvest than Bifera accessions, whereas both groups of Bifera accessions were significantly higher yielding ($P < 0{\cdot}05$) in the second harvest and in total annual DMY ($P < 0{\cdot}05$, Table 3). In the third harvest, a similar trend in yield was observed as in the second harvest. There were some significant differences among accessions within the types, but differences between ecotypes and landraces within Communis types and between landraces and cultivars within Bifera types were small. Over all growth cycles, Bifera accessions were more ($P < 0{\cdot}05$) vigorous than Communis accessions. There were clear differences between sainfoin accessions in their tendency to flower in the seeding year (SYF) with an almost complete absence of flowering of Communis accession and strong, somewhat variable flowering of Bifera accession.

Across all three sites and two harvests, Communis accessions had higher ($P < 0{\cdot}05$) CP concentrations than Bifera accessions (Table 4). The accessions Premier and Visnovsky had the highest and the lowest CP

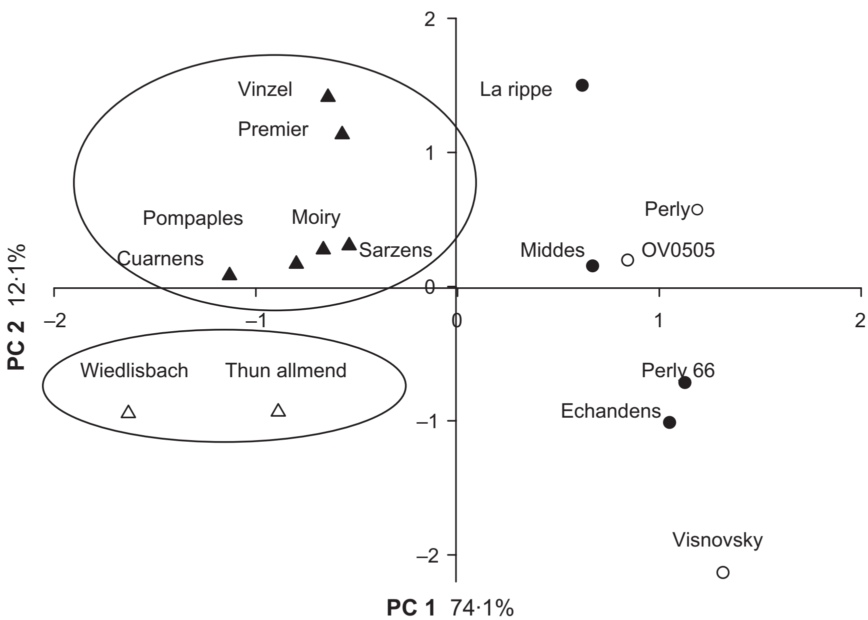

Figure 1 Plot of the first two PC score vectors for the data set on morphological and phenological characterization. Open circles, Bifera cultivars; closed circles, Bifera landraces; open triangles, Communis ecotypes; closed triangles, Communis landraces.

concentration respectively. Across sites and harvests, both groups of Bifera accessions showed lower CT concentrations than both Communis groups ($P < 0{\cdot}05$). Wiedlisbach and Visnovsky had the highest and lowest CT concentration respectively. The two Communis groups were lower in NDF ($P < 0{\cdot}05$) and ADF ($P < 0{\cdot}05$) than the Bifera groups. Despite the clear differences between the four groups in CP, NDF and ADF, *in vitro* organic matter digestibility was similar ($P > 0{\cdot}05$) for Communis and Bifera types.

Concentration of minerals (g kg^{-1} DM) ranged between 9·8 and 12·1 for Ca, 3·1 and 3·5 for P, 1·9 and 2·2 for Mg and 15·7 and 17·9 for K (data not shown in tables). Concentration of Na was below the detection limit of 0·2 g kg^{-1} DM. Concentration of trace elements (mg kg^{-1} DM) ranged between 48·8 and 70·3 for Fe, 26·8 and 30·7 for Zn, 24·4 and 29·1 for Mn and 6·4 and 7·8 for Cu. There were no significant differences ($P > 0{\cdot}05$) between accessions or types, except for Fe, where Bifera accessions showed lower ($P < 0{\cdot}05$) concentrations than Communis accessions (55·6 vs. 63·5 mg kg^{-1} DM).

Communis ecotypes showed higher ($P < 0{\cdot}05$) gas production with the addition of PEG (GP_P) and a higher PEG effect on GP than the other groups. This was mainly the result of the accession Wiedlisbach, where the PEG effect on GP was higher ($P < 0{\cdot}05$) than in all other accessions. Although there were differences ($P < 0{\cdot}05$) in the PEG effect on GP among sainfoin accessions, these differences were small, and the PEG effect hardly exceeded 6%. The control legume *Trifolium pratense*, which presented a very low CT concentration (<5 g kg^{-1} DM), had a higher ($P < 0{\cdot}01$) GP (51·5 mL) within 24 h when compared to the sainfoin accessions (45·4 mL on average).

Effect of harvest

There was a significant effect of harvest (Table 5) on most compositional variables as well as on digestibility, gas production and NEL concentration. Furthermore, for all variables investigated, except for PEG effect, harvest × group interactions ($P < 0.001$) were very highly significant. In Communis groups, higher CP and CT concentrations ($P < 0{\cdot}05$) were found in the second harvest compared with the first harvest. In Bifera accessions, CP concentration was not affected by harvest ($P > 0{\cdot}05$), and only in Bifera landraces, higher CT

Table 5 Chemical composition of grouped sainfoin accessions in the two harvests averaged across 3 sites (n = 135 per harvest, Experiment 2).

Harvest/Group	OM	CP	NDF	ADF	CT	IVDOM	GP_n	GP_p	PEG	NEL
First harvest										
Average	941^{A}*	166^{B}	408^{A}	362	$50{\cdot}7^{B}$	$63{\cdot}5^{A}$	$49{\cdot}0^{A}$	$51{\cdot}1^{A}$	$4{\cdot}59^{B}$	$5{\cdot}98^{A}$
Communis ecotype	941^{ab}	169^{b}	407^{ab}	366^{ab}	$55{\cdot}0^{cd}$	$63{\cdot}0^{a}$	$48{\cdot}8^{a}$	$51{\cdot}8^{a}$	$6{\cdot}16^{abc}$	$6{\cdot}00^{a}$
Communis landrace	943^{a}	169^{b}	416^{a}	370^{a}	$49{\cdot}3^{e}$	$63{\cdot}2^{a}$	$48{\cdot}2^{a}$	$49{\cdot}5^{b}$	$2{\cdot}73^{d}$	$5{\cdot}92^{a}$
Bifera landrace	943^{a}	164^{b}	399^{b}	350^{cd}	$49{\cdot}8^{e}$	$63{\cdot}5^{a}$	$49{\cdot}1^{a}$	$50{\cdot}9^{ab}$	$3{\cdot}38^{cd}$	$5{\cdot}99^{a}$
Bifera cultivar	939^{b}	167^{b}	393^{b}	352^{bc}	$52{\cdot}8^{de}$	$64{\cdot}4^{a}$	$49{\cdot}1^{a}$	$51{\cdot}1^{a}$	$5{\cdot}09^{bc}$	$6{\cdot}03^{a}$
Second harvest										
Average	933^{B}	171^{A}	389^{B}	356	$66{\cdot}7^{A}$	$56{\cdot}9^{B}$	$42{\cdot}2^{B}$	$44{\cdot}9^{B}$	$6{\cdot}38^{A}$	$5{\cdot}36^{B}$
Communis ecotype	931^{d}	180^{a}	357^{c}	336^{de}	$80{\cdot}1^{a}$	$56{\cdot}4^{bc}$	$42{\cdot}5^{bc}$	$46{\cdot}2^{c}$	$8{\cdot}48^{a}$	$5{\cdot}48^{bc}$
Communis landrace	932^{d}	181^{a}	361^{c}	334^{e}	$73{\cdot}5^{b}$	$57{\cdot}8^{b}$	$43{\cdot}5^{b}$	$45{\cdot}9^{c}$	$5{\cdot}75^{bc}$	$5{\cdot}54^{b}$
Bifera landrace	935^{c}	165^{b}	417^{a}	375^{a}	$57{\cdot}2^{c}$	$56{\cdot}6^{bc}$	$41{\cdot}9^{c}$	$44{\cdot}1^{d}$	$6{\cdot}16^{ab}$	$5{\cdot}28^{c}$
Bifera cultivar	933^{cd}	163^{b}	419^{a}	381^{a}	$57{\cdot}2^{cd}$	$56{\cdot}3^{c}$	$41{\cdot}8^{c}$	$43{\cdot}9^{d}$	$5{\cdot}41^{bc}$	$5{\cdot}26^{c}$
Average SE of harvest × group LS mean	0·9	2·4	3·7	3·4	1·01	0·40	0·44	0·42	0·598	0·047
P values										
Harvest	<0·001	<0·001	<0·001	0·137	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001
Group	<0·001	<0·001	<0·001	<0·001	<0·001	0·150	0·753	0·007	<0·001	0·033
Harvest × group	<0·001	<0·001	<0·001	<0·001	<0·001	0·001	0·001	<0·001	0·087	<0·001

OM, organic matter (g kg^{-1} DM); CP, crude protein (g kg^{-1} DM); NDF, neutral detergent fibre (g kg^{-1} DM); ADF, acid detergent fibre (g kg^{-1} DM); CT, condensed tannins (g kg^{-1} DM); IVDOM, *in vitro* digestibility of organic matter (%); GP_n, gas production without PEG (mL 24 h^{-1}); GP_p, gas production with PEG (mL 24 h^{-1}); PEG, increase in gas production because of PEG (% of GP_n); NEL, net energy lactation (MJ kg^{-1} DM).

*Within a column, means followed by different superscripts differ significantly ($P < 0{\cdot}05$). Capital letters indicate significant differences among harvests, and lower case letters indicate significant differences among groups of accessions.

concentrations ($P < 0{\cdot}05$) were found in the second harvest. Further, IVDOM and gas production were lower ($P < 0{\cdot}05$) in the second harvest than in the first harvest. Lower ($P < 0{\cdot}05$) PEG effects on GP and higher NEL values were observed for the first harvest compared with the second harvest. Except for K and P, mineral and trace element concentrations were higher ($P < 0{\cdot}05$) in the second harvest than in the first harvest (data not shown in tables). For most of the variables investigated, differences between Communis and Bifera groups were much more pronounced in the second harvest as compared to the first harvest. This explains the repeatedly occurring significant harvest × group interactions.

Effect of site

There were clear site effects (mostly at $P < 0{\cdot}001$) for the variables investigated but no site × group interactions ($P > 0{\cdot}05$ for all variables; Table 6), indicating a remarkable environmental stability of the differences among groups. Total DMY (not shown in Table 6) also differed ($P < 0{\cdot}001$) among sites, with an average of 13·6 t ha^{-1} obtained for Thun compared with 11·4 and 11·5 t ha^{-1} for Ellighausen and Reckenholz respectively. At Thun and Ellighausen, NDF, ADF and CT concentrations were lower ($P < 0{\cdot}05$) than at Reckenholz, whereas GP, IVOMD and NEL concentration were higher ($P < 0{\cdot}05$) in samples from Thun and Ellighausen than in samples from Reckenholz.

Discussion

The results revealed a considerable variation among accessions in most phenological, morphological and chemical variables, and this variation was remarkably stable across environments, but clearly differed with time.

Table 6 Chemical composition of the grouped sainfoin accessions at the three sites averaged across harvests 1 and 2 (n = 90 per site, Experiment 2).

Site/Group	OM	CP	NDF	ADF	CT	IVDOM	GP_n	GP_p	PEG	NEL
Thun										
Average	937^{AB}*	172	388^{B}	350^{B}	$55{\cdot}2^{C}$	$61{\cdot}3^{A}$	$48{\cdot}0^{A}$	$49{\cdot}7^{A}$	$3{\cdot}82^{B}$	$5{\cdot}91^{A}$
Communis ecotype	935^{abc}	178^{abc}	367^{d}	339^{e}	$65{\cdot}7^{ab}$	60·0	48·2	$50{\cdot}5^{a}$	$4{\cdot}25^{c}$	$5{\cdot}97^{a}$
Communis landrace	937^{abc}	180^{a}	374^{d}	340^{e}	$58{\cdot}2^{de}$	62·1	48·0	$49{\cdot}8^{ab}$	$3{\cdot}58^{c}$	$5{\cdot}96^{a}$
Bifera landrace	940^{ab}	167^{bcde}	400^{bc}	354^{de}	$50{\cdot}7^{f}$	61·4	47·4	$49{\cdot}2^{ab}$	$4{\cdot}24^{c}$	$5{\cdot}83^{a}$
Bifera cultivar	937^{abc}	171^{abcde}	395^{bc}	360^{cd}	$51{\cdot}5^{f}$	61·5	47·0	$49{\cdot}0^{ab}$	$4{\cdot}69^{bc}$	$5{\cdot}82^{a}$
Ellighausen										
Average	934^{B}	168	391^{B}	344^{B}	$58{\cdot}7^{B}$	$60{\cdot}9^{A}$	$46{\cdot}2^{B}$	$48{\cdot}5^{A}$	$5{\cdot}37^{AB}$	$5{\cdot}76^{B}$
Communis ecotype	935^{bc}	178^{ab}	377^{cd}	337^{e}	$66{\cdot}1^{ab}$	60·9	45·8	$49{\cdot}3^{ab}$	$8{\cdot}13^{ab}$	$5{\cdot}84^{a}$
Communis landrace	934^{bc}	175^{abcd}	384^{cd}	340^{e}	$62{\cdot}3^{bcd}$	60·6	46·3	$48{\cdot}4^{ab}$	$4{\cdot}32^{c}$	$5{\cdot}79^{a}$
Bifera landrace	935^{bc}	166^{bcde}	398^{bc}	345^{de}	$53{\cdot}9^{ef}$	61·2	46·2	$48{\cdot}4^{ab}$	$4{\cdot}66^{bc}$	$5{\cdot}71^{a}$
Bifera cultivar	933^{c}	165^{cde}	397^{bc}	348^{de}	$54{\cdot}9^{ef}$	61·7	46·2	$48{\cdot}2^{ab}$	$5{\cdot}46^{bc}$	$5{\cdot}74^{a}$
Reckenholz										
Average	940^{A}	164	415^{A}	383^{A}	$62{\cdot}2^{A}$	$58{\cdot}3^{B}$	$42{\cdot}6^{C}$	$45{\cdot}8^{B}$	$7{\cdot}28^{A}$	$5{\cdot}35^{C}$
Communis ecotype	938^{abc}	167^{bcde}	402^{bc}	377^{abc}	$70{\cdot}9^{a}$	58·2	42·9	$47{\cdot}3^{bc}$	$9{\cdot}58^{a}$	$5{\cdot}41^{b}$
Communis landrace	941^{a}	171^{abcd}	407^{b}	376^{bc}	$63{\cdot}7^{bc}$	58·8	43·1	$44{\cdot}9^{c}$	$4{\cdot}81^{bc}$	$5{\cdot}43^{b}$
Bifera landrace	942^{a}	160^{e}	426^{a}	389^{ab}	$55{\cdot}9^{ef}$	57·6	42·8	$45{\cdot}0^{c}$	$5{\cdot}42^{bc}$	$5{\cdot}36^{b}$
Bifera cultivar	939^{abc}	160^{de}	425^{a}	392^{a}	$58{\cdot}6^{cde}$	57·9	43·2	$45{\cdot}5^{c}$	$5{\cdot}50^{bc}$	$5{\cdot}37^{b}$
Average SE of Site × group LS mean	1·3	3·5	4·8	4·4	1·24	0·53	0·54	0·51	0·755	0·057
P value										
Site	0·034	0·131	<0·001	<0·001	0·002	0·001	<0·001	<0·001	0·048	<0·001
Group	<0·001	<0·001	<0·001	<0·001	<0·001	0·150	0·753	0·007	<0·001	0·032
Site × group	0·088	0·756	0·683	0·562	0·738	0·054	0·876	0·652	0·089	0·970

OM, organic matter (g kg^{-1} DM); CP, crude protein (g kg^{-1} DM); NDF, neutral detergent fibre (g kg^{-1} DM); ADF, acid detergent fibre (g kg^{-1} DM); CT, condensed tannins (g kg^{-1} DM); IVDOM, *in vitro* digestibility of organic matter (%); GP_n, gas production without PEG (mL 24 h^{-1}); GP_p, gas production with PEG (mL 24 h^{-1}); PEG, increase in gas production because of PEG (% of GP_n); NEL, net energy lactation with PEG (MJ kg^{-1} DM).

*Within a column, means followed by different superscripts differ significantly ($P < 0{\cdot}05$). Capital letters indicate significant differences among sites, and lower case letters indicate significant differences among groups of accessions.

Effect of accession

The variability in morphological and phenological traits allowed a clear arrangement of the 15 accessions. As in previous reports (Sheldrick *et al*., 1995; Delgado *et al*., 2008; Piano and Pecetti, 2010), two main botanical forms within this species were the Communis type, which provides only one flowering cut per year with regrowth remaining mostly vegetative, and the Bifera type providing two to three flowering harvests per year. The present study permitted a further categorization of Communis accessions into ecotypes and landraces. A distinction of Bifera accessions into these two groups was not evident in Experiment 1. Therefore, Bifera accessions were arranged based on their origin into landraces and cultivars (commercial and candidate cultivars). Using cluster analysis, Delgado *et al.* (2008), in a study with 44 sainfoin accessions, were able to further separate both Bifera and Communis accessions into three groups each. This confirms the heterogeneity that sainfoin germplasm may exhibit.

Trends observed between accessions in the morphological and phenological characterization could help to explain some of the differences among accessions in DMY and chemical composition. The longer stem lengths found in Bifera accessions, compared with Communis accessions, translated into a higher total DMY. This was particularly evident with the Visnovsky accession that outperformed most other accessions in total DMY. Total DMY obtained in this study ranged from 10·7 to 14·6 t ha^{-1} (12·5 t ha^{-1} on average) and was within the range found in previous studies (Häring *et al*., 2008; Liu *et al*., 2008). However, it should be noted that the results reported here refer only to the second year of the stand. In the third year of the stand, total DMY was considerably lower, ranging from 5.9 to 8.9 t ha^{-1} among the accessions (data not shown), and pointing to the need to improve persistency of sainfoin under local field conditions. The total DMY of individual sainfoin accessions reflected the level of human intervention as described by Boller and Greene (2010), with cultivars being the most productive, landraces being intermediate and ecotypes being lowest in total DMY.

Ranges found for CP and fibre concentrations were within those previously reported by Liu *et al.* (2008). Communis type accessions had higher CP and CT, but lower fibre concentrations compared with Bifera accessions. As a rule, nutritive value in legumes tends to be higher in late- than in early maturing cultivars when they are harvested late in the growing season (Porqueddu, 2001), because early maturing cultivars start to bloom earlier, and the proportion of stems is therefore higher. Borreani *et al.* (2003) reported that at the full flowering stage, sainfoin stems constitute the bulk of the material harvested. Accordingly, the extra total DMY found in Bifera accessions, compared with Communis accessions, largely consisted of stem tissue material that has a lower CP concentration and a higher fibre concentration compared with leaf material (Häring *et al*., 2007). Unexpectedly, the differences in fibre concentration were not associated with differences in gas production or *in vitro* digestibility.

One of the most prominent features of sainfoin is its concentration of CT, which was in a range of 47–80 g kg^{-1} DM. This is considered to be high enough for being beneficial in terms of ruminal bypass protein and other properties such as the inhibition of gastrointestinal nematodes (Heckendorn *et al*., 2006). The mode of action is mainly based on the formation of complexes with dietary proteins and also rumen bacteria, which could limit protein degradability, GP and fibre digestibility (Aerts *et al*., 1999; Min *et al*., 2003). The CT concentrations found were within the range reported by others (Scharenberg *et al*., 2007; Häring *et al*., 2008; Lorenz *et al*., 2010). The use of PEG, a specific tannin-complexing agent, showed the expected increases in GP across all accessions. The PEG effect was expected to be particularly large in high-CT accessions. Consistent with that, the accession Wiedlisbach could be distinguished from all the other accessions in extra GP with PEG. However, even in the presence of PEG, GP from sainfoin samples was lower than GP from *T. pratense*, the low-CT control legume. This suggests that the lower NEL concentration of sainfoin as compared to *T. pratense* (5·5–5·8 vs. 6·0–6·4 MJ kg^{-1} DM; Agroscope Liebefeld-Posieux, 2009) is not only the result of the higher CT concentration but probably also related to the higher fibre concentration of sainfoin.

Effect of harvest

Sainfoin herbage harvested in the first and the second harvest differed significantly in chemical composition. Additionally, there was a significant harvest × group interaction in many variables, reflecting the clear response of Communis types and the lack of response of Bifera types to harvest time. Consistent with the present findings, Liu *et al.* (2008) reported increased CP and decreased fibre concentrations from the first to the second harvest during the first full harvest year in a Communis accession of sainfoin. The different growth features of the sainfoin types may explain the observed differentiation, as shown by the significant interaction, in CP and fibre from the first to the second harvest.

Communis and Bifera types exhibited different temporal dynamics in CT concentration, with markedly higher CT concentrations in the second harvest than in the first harvest for the Communis accessions, and little difference for the Bifera accessions. Häring *et al.* (2007), modelling the dynamics of CT concentration in different plant parts of two Bifera accessions, reported a doubling of CT concentration in leaves but found no statistical evidence for changes in CT concentrations in the total above-ground biomass because of the diluting effect of the increasing proportion of the low-tannin stems. This is consistent with the present findings, where Bifera accessions showed relatively smaller increases in CT concentration compared with Communis accessions. The increase in CT found in the second harvest in all sainfoin groups might also be associated with the higher average temperature during the second growth period. For birdsfoot trefoil (*Lotus corniculatus*), another tannin-containing legume, it has been demonstrated that CT concentration increases during the warmer months (Cassida *et al.*, 2000). The mean temperature difference between the growth periods preceding the first and the second harvest was about 4°C.

Concerning *in vitro* digestibility and gas production, any effect of the marked increase in CT from the first to the second harvest for Communis accessions compared with Bifera accessions seems to have been counterbalanced by a corresponding decrease in fibre concentration and an increase in CP concentration, eventually resulting in similar values in both harvests. Concentration of NEL was even higher in Communis landraces than in Bifera accessions. This observation is of particular interest because it indicates that in sainfoin, higher CT concentrations are not necessarily associated with a lower forage value.

Effect of site

A significant site effect was observed in most variables, indicating a considerable influence of site on growth and nutritional composition of sainfoin. Chemical composition, *in vitro* digestibility and NEL concentration differed considerably between Reckenholz and Thun, whereas values from Ellighausen were intermediate or similar to those from Thun. Furthermore, total DMY was higher at Thun than at Ellighausen and Reckenholz. It is well known that the cultivation site may affect yield and composition of forage plants. Highly tanniniferous tropical legumes, grown on two contrasting soils and exposed to different levels of water stress, were found not only to differ extremely in their DMY (Tiemann *et al.*, 2009) but also in chemical composition and CT concentration (Tiemann *et al.*, 2010). Although soils were not analysed in detail in the present study, the lower total DMY from the Ellighausen and Reckenholz sites compared with Thun might be related to differences in soil fertility. Climatic conditions and altitude were quite similar, except for lower rainfall at Ellighausen, making these factors less likely to have been important.

Ranking of the different sainfoin groups in the individual variables, although not exactly the same across the three sites, showed a lack of significance of site × group interaction. The implication of this is that, despite the clear changes induced by different environments on the variables measured, differences between sainfoin types determined at one site can be expected to occur at other sites as well.

Conclusions

The results of the present study clearly demonstrate that historical landraces and newly collected ecotypes expand the range of genetic variation available for sainfoin selection and breeding. The various accessions of the Swiss collection investigated here exhibited considerable variability in their morphological and phenological traits, allowing them to be grouped into single- and multiple flowering accessions. This grouping explained some of the variation observed in agronomic yields and chemical composition among accessions and facilitates the selection of accessions with desired properties (high yield, high forage value or high tannin concentration) to be developed further. Marked differences in agronomic yields and tannin concentrations in sainfoin types, caused by time of harvest, imply that when aiming for forage with high CT concentration, the leafy regrowth (second harvest) of Communis accessions is the most promising option. Despite clear significant changes induced by site, differences between sainfoin accessions determined at one site could be expected at other sites as well.

Acknowledgments

The authors are grateful to the Group of Analytical Chemistry of Agroscope Liebefeld-Posieux for the help provided in the analysis of chemical composition of the samples. This study was supported by a grant from the Swiss Grassland Society (Arbeitsgemeinschaft zur Förderung des Futterbaues, AGFF), Zurich, Switzerland, and would not have been possible without support of the Swiss Federal Department of Economic Affairs through its National Action Plan 'Diversity in Agriculture Campaign Plants'.

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