Herbage dry-matter production and forage quality of three legumes and four non-leguminous forbs grown in single-species stands

A. Elgersma*, K. Søegaard† and S. K. Jensen‡

- *????, Wageningen, The Netherlands, †Department of Agroecology and Environment, Faculty of Agricultural Science, Aarhus University, Tjele, Denmark, ‡Department of Animal Sciences, Faculty of Agricultural Science,
- 2 Aarhus University, Tjele, Denmark

Abstract

Agronomic data on most broad-leaved species of grasslands are scarce. The aim of this study was to obtain novel information on herbage DM yield and forage quality for several forb species, and on species differences and seasonal patterns across harvests and in successive years. Four non-leguminous forbs [salad burnet (Sanguisorba minor), caraway (Carum carvi), chicory (Cichorium intybus) and ribwort plantain (Plantago lanceolata)] and three leguminous forbs [vellow sweet clover (Melilotus officinalis), lucerne (Medicago sativa) and birdsfoot trefoil (Lotus corniculatus)] and a perennial ryegrass-white clover mixture were investigated in a small-plot cutting trial in Denmark during 2009 and 2010. Plots were harvested four times per year. On average, annual herbage yield was highest for lucerne (15-4 DM ha⁻¹) and grass-white clover (15.4 t DM ha⁻¹), and lowest for salad burnet $(4.6 \text{ t DM ha}^{-1})$ and yellow sweet clover (3.9 t DM ha⁻¹). Ribwort plantain and lucerne had the highest concentrations of acid detergent fibre (339 and 321 g kg⁻¹ DM respectively) and lignin (78 and 67 g kg⁻¹ DM respectively); contents in other species were similar to grass-white clover (275 and 49 g kg⁻¹ DM respectively). No common feature was found within the functional groups of non-leguminous forbs and leguminous forbs, other than higher crude protein contents (198-206 g kg 1 DM) in the legumes. DM yield and fibre content were lowest in October. Digestibility declined with higher temperature and increasing fibre content. Results are discussed in terms of the

Correspondence to: A. Elgersma, PO Box 323, 6700 AH Wageningen, The Netherlands.
E-mail: anjo.elgersma@hotmail.com

Received 4 August 2013; revised 28 October 2013

potential of forbs to contribute to forage resources in farming practice.

Keywords: functional groups, herbs, forbs, legumes, forage quality, seasonal variation

Introduction

Herbaceous species of grasslands in temperate climate zones can be classified into three broad plant functional groups: grasses, forage legumes and nonleguminous forbs (Schellberg and Pontes, 2012). Sown grasslands, particularly when under intensive management, are usually based on simple mixtures of grass cultivars, often of only one species, or a grass-legume association. Non-leguminous forbs are generally not included in seeds mixtures for agricultural grasslands, except for special situations such as organic farms (Younie, 2012) or agri-environmental measures. In contrast, semi-natural grasslands include a greater range of species, including legumes and other forbs, and in some cases, these are of high feed value (Jeangros and Thomet, 2004). Yield and herbage quality of semi-natural grasslands also vary, depending on species composition, at successive growth cycles during the growing season (Michaud et al., 2012). Perceived benefits of forbs in grassland swards have been widely reported, although often based on limited evidence (Voisin, 1959; Foster, 1988; Smidt and Brimer, 2005). More recently, multispecies swards have been advocated as having potential to provide increased availability of nutrients supplied by forbs. These include not only essential microminerals that are present in chicory (Cichorium intybus L.) and ribwort plantain (Plantago lanceolata L.) (Pirhofer-Walzl et al., 2011; Lindström et al., 2013), but also enhanced vitamin and fatty acids concentrations beneficial for animal diets (Elgersma et al., 2012) and for the sensory properties and physical characteristics of animal-derived products

Journal Code Manuscript No. Species 12 1 0 4

WILEY No. of pages: 13

CE: Shantle PE: Suiitha

(Moloney et al., 2008). However, there is relatively little information on the forage yield potential and feeding value of most non-leguminous forb species of grasslands. In parts of Europe, such as in areas with mountain pastures, there is acceptance that some forbs are of high value, but in lowland areas such as Denmark and the Netherlands, especially where there is a history of intensive use, many advisors and farmers expect forbs to have negative effects on herbage yield. There is therefore a knowledge gap in terms of quantitative data on the yield and quality of forbs in lowland regions with intensive systems, and results from extensively used (semi-) natural grasslands may not be applicable to intensive high-input farming systems.

If benefits of forbs could be demonstrated, for example in terms of improved animal health, feeding value and quality of the product (Hopkins and Wilkins, 2006), there will be need for further agronomic information on production characteristics and forage quality. Forage yield data have been published for chicory (e.g. Collins and McCoy, 1997; Kunelius and MacRae, 1999; Li and Kemp, 2005) and to a lesser extent for ribwort plantain (Sanderson et al., 2003a.b). These two species are high yielding relative to many other forbs (Labreveux et al., 2004) and are included in some commercial forage mixtures, for instance, in New Zealand dairy pastures (Glassey et al., 2013), but agronomic information on other forbs is sparse. In particular, quantitative data from field trials carried out during the growing season on potential dry-matter (DM) yield and feed value of non-leguminous forbs relative to grasses and the main forage legumes, and in relation to environmental factors, are lacking

We hypothesized that yield and forage quality of some forbs might be similar to that of a perennial ryegrass grass-white clover (Lolium perenne-Trifolium repens) mixture. The aim of this study therefore was to obtain information on yield and herbage quality for several leguminous and non-leguminous forb species in comparison with a grass-white clover mixture, and to investigate species differences across eight successive harvest dates.

Materials and methods

Experimental site, species and establishment

The experiment was established after ploughing and cultivation in spring 2008 at the Research Farm Foulumgaard, Aarhus University, central Jutland, Denmark (56°29'N, 9°34'E; 51 m a.s.l.). The loamy sand soil is a typic Hapludult (Soil Survey Staff, 1998) which consists of 7.7% clay, 10% silt, 48% fine sand, 33% coarse sand and 1.6% carbon. Soil pH KCI was 5.9, and it had 60 mg kg⁻¹ exchangeable K and 21 mg kg⁻¹ extractable P in the 0-15 cm layer.

Forage species were undersown with spring barley (Hordeum vulgare L.), sown at 25 kg ha-1 and a sowing depth of 0.5-1 cm. Pure stands were established of five non-leguminous forb species, four legumes and a mixture (0.85: 0.15 by seed weight) of perennial ryegrass and white clover ('Blanding 22', a recommended Danish seed mixture for grasslands). The non-leguminous forbs were salad burnet (Sanguisorba minor Scop.), caraway (Carum carvi L.), chicory, ribwort plantain and chervil [Anthriscus cerefolium (L.) Hoffm.] The legumes were yellow sweet clover [Melilotus officinalis (L.) Pall.], lucerne (Medicago sativa L.), birdsfoot trefoil (Lotus corniculatus L.) and fenugreek (Trigonella foenum-graecum L.). All seeds were obtained from commercial seed suppliers.

The experimental design was a randomized block with two replications. Net plot size was $1.5 \text{ m} \times 9 \text{ m}$. After the harvest of the spring barley at maturity in August 2008, the experimental plots were cut on October 25. Fenugreek did not survive the winter, and on 16 April 2009, the bare fenugreek plots were oversown with borage (Borago officinalis L.) in replicate 1 and viper's bugloss (Echium vulgare L.) in replicate 2. As there was poor growth of chervil after the first harvest in 2009, this species was disregarded in statistical analyses. (Results of forage quality parameters of borage, viper's bugloss and chervil are summarized in a supplementary file.) Statistical analyses were therefore 4 carried out with four non-leguminous forbs, three leguminous forbs and the grass-white clover mixture.

Agronomic management

One replicate was fertilized with cattle slurry, applied with hoses in spring (17 April 2009 and 15 April 2010) and after the second cut (10 July 2009 and 15 July 2010) at 40.5 and 30.8, 23.9 and 20.5 t ha-1 respectively. The dates and amounts reflect typical slurry applications on non-organic farms in the region. The other replicate received only potassium sulphate in amounts that provided K comparable with that in the slurry: 200 kg K ha-1 was applied on 10 July 2009, and 100 kg K ha^{-1} on 7 April 2010 and 16 July 2010 (after the second cut). As P was not limiting, no P was applied. Thus, the main difference between the potassium sulphate-treated plots and the slurry-treated plots was the additional N in slurry (ca. 190 kg ha⁻¹ year⁻¹ total N in slurry, of which ca. 114 kg ha⁻¹ was NH₄-N). As replicate was confounded with slurry, a model was chosen with replicate (slurry) as fixed effect. Irrigation was applied on 9 and 29 June 2009 (38 and 54 mm respectively) and on 15

July 2010 after the second cut (15 mm following slurry application) to prevent drought stress.

Swards were cut with a forage harvester (J Haldrup A/S, Løgstør, Denmark) to a residual stubble height of 7 cm. Harvesting dates in 2009 were 29 May, 9 July, 21 August and 23 October, and in 2010, 31 May, 13 July, 19 August and 21 October. These dates corresponded with the standard dates for grass harvesting of typical four-cut systems in Denmark, which are based on calender date. Weather data were recorded daily at the Foulumgaard weather station within 500 m of the plot area.

Sample processing and chemical analyses

After cutting, the herbage was weighed and subsamples taken. One ca. 200 g subsample was dried at 60°C for 48 h in an air-forced oven, and used to determine DM content and for all chemical analyses. Ash was determined after combustion for 6 h at 525°C, and N content was determined according to the Dumas method (Hansen, 1989). A Fiber-Tec system was used to determine neutral detergent fibre (NDF) (Mertens, 2002), acid detergent fibre (ADF) and acid detergent lignin (ADL) (van Soest, 1963). In vitro organic matter digestibility (IVOMD) was determined in rumen liquor according to Tilley and Terry (1963). Sampling of rumen liquor complied with animal welfare regulations. A second sample was taken from each cut in both years and freeze-dried to provide material for measuring fatty acid (FA) concentration (Jensen,

The botanical composition of the grass-white clover sward was not determined. In the forb plots, unsown species were excluded from the subsamples used for chemical analyses. Chervil, borage and viper's bugloss samples were analysed when the amount of herbage was sufficient.

Statistical analysis

Analysis of variance procedures were applied using the MIXED procedures of SAS (Version 9) (SAS Insti-5 tute, 2001; Littell et al., 2006). There were eight 'species' (the seven forb species plus the grass-clover mixture) and four harvests per year. Crude protein (CP) was calculated as 6.25 × N. The category 'other compounds' was calculated as 1000 - (NDF + CP + FA + Ash). Lignin content equalled ADL, and cellulose was calculated as ADF-ADL and hemicellulose as NDF-ADF. The experimental design was a randomized complete block with two replications which were, however, confounded with management; hence statistically, sample size was N = 1. Therefore (Cochran and Cox, 1957), yield and quality parameters were

evaluated with a model that included a fixed main effect of replicate (block = slurry) (λ_b) to account for differences in fertilization regime and possible effects of N and P on yield and quality:

$$Y_{bscy} = \mu + \alpha_s + \beta_c + (\alpha\beta)_{sc} + \delta_y + \lambda_b + (\delta\lambda)_{yb} + A_{bs} + B_{bsc} + C_{sy} + D_{bsy} + E_{bscy}$$

where y_{bscy} = The recorded value for species s in cut c of replicate (block) b in year y

Greek letters denote fixed effects. Capital Latin letters denote random effects. Lower case Latin letters identify the effects and observations.

The following four effects were considered to be random effects: replicate \times species, replicate \times species \times cut, species \times year and replicate \times species \times year. Because the year \times replicate effect $(\delta \lambda)_{yb}$ was not significant for any of the parameters in a first analysis, this interaction term was deleted and the analysis was repeated. The interaction species \times cut $(\alpha\beta)_{sc}$ was significant and was kept in the model as a fixed effect. The interaction between replicate and some other effects were small, and they were kept in the model as random effects to take into account the random variation between plots and to take into account certain correlations that must be expected because of the design structure (e.g. same plot measured in both years). When analysing experiments with a sample size N = 1, anova can be performed if some interaction terms are random or absent and assuming that those random or absent terms have no true fixed effect (Cochran and Cox, 1957). These assumptions were

Differences detected among main effects and interactions were assessed using the PDIFF option in the least-squares means statement. Regression analyses were conducted between yield, quality parameters and weather variables. All tests of significance were made at P = 0.05.

Results

Species differences

In the cut taken in the establishment year (October 2008; data not shown), there was poor growth of salad burnet and caraway, with insufficient herbage for a yield determination. The DM yield of chervil was 0.65 t ha^{-1} and yields of the remaining species treatments ranged from 1.5 t DM ha-1 in ribwort plantain to 2.2 t DM ha⁻¹ in lucerne.

Annual DM yield in 2009 ranged from 4 t ha⁻¹ for caraway to 16 t ha-1 for lucerne. In 2010, these same species yielded 7 and 15 t ha⁻¹ respectively. In 2010,

yellow sweet clover produced insufficient herbage for a yield determination, and in the fourth cut, there was no measureable yields from either salad burnet or birdsfoot trefoil. In most cases, however, the amount of herbage was sufficient for the determination of quality analyses.

The mean values for DM yield and herbage chemical composition of the seven forb species and the grass-clover mixture are shown in Table 1. There were significant differences among the species for most parameters investigated. There were no significant block differences. The average DM yield was highest for lucerne, followed by the grass-clover mixture, and was lowest for salad burnet and yellow sweet clover (P < 0.001). The grass-clover mixture had the highest in vitro organic matter digestibility. There was no relation between yield and IVOMD based on species means (Table 1).

Ribwort plantain and lucerne had the highest concentrations of NDF, ADF and ADL (P < 0.001, Table 1). Birdsfoot trefoil had low NDF and ADF concentrations, but a high ADL concentration and thus high lignification of the cell wall, as well as a low ash content. The apparently higher hemicellulose proportion of the cell walls in the grass-clover mixture, compared with non-leguminous forbs, was not significant due to species x cut interactions (Table 1).

The highest ash concentration was found in chicory (P < 0.001). There were no overall relationships between NDF and ash contents, or any effect of functional group. The CP concentration was highest in the three legume species and in the grass-clover mixture, and lowest in chicory and plantain (P < 0.001). The concentration of 'other compounds' was significantly higher in salad burnet than in all other species, and also higher in chicory and caraway than in legume species and the mixture (P < 0.001).

The concentration of CP in the legumes (and the grass-clover mixture) exceeded that in the nonleguminous forbs, while the opposite was found for 'other compounds'. No other differences in DM composition or quality were found between the functional groups of legumes and non-leguminous forbs. Within each functional group, significant differences between individual species occurred for all traits except for CP in the legumes functional group (Table 1).

Concentrations of NDF, ADF, ADL, CP, 'other compounds' and ash, and IVOMD of chervil, borage and viper's bugloss are presented in Appendix 1.

Seasonal fluctuation

Differences between harvests (P < 0.001) were found for all parameters (Table 1). Forage DM yields and concentrations of NDF, ADF and ADL were lowest in the fourth harvest. Concentrations of ash and CP, as well as digestibility, increased from the second cut onwards.

Weather variables during spring growth and during each regrowth interval are shown in Figure 1. The winter of 2009-10 was severe and spring growth started late. The average temperature in April 2010 was only 6.5°C, whereas in April 2009, it was 9.4°C. Mean temperatures during the 40 d preceding the date of the first cut were 8.4°C in 2010 and 10.3°C in 2009 (Figure 1a). In 2010, there was a cold spring with retarded growth, and temperatures fluctuated greatly during the last 2 weeks of the first regrowth period (Figure 1b). During the second regrowth period, temperatures did not differ between the 2 years (Figure 1c) except the first 10 d. During the third regrowth period, average weather conditions were also similar (Figure 1d).

Herbage DM production

Annual yield was lower in 2010 than in 2009 for salad burnet (0.92), lucerne (0.91), plantain (0.79), birdsfoot trefoil (0.75), grass-white clover (0.75) and chicory (0.53). In contrast, caraway yielded almost twice as much in 2010 than in 2009. Due to this variability, there was no overall effect of year (Table 1).

The seasonal growth pattern was very different between years: in 2009, the first cut produced the greatest yield, whereas in 2010, the greatest yield was at second cut for all species except caraway. For most species, the DM yield of the first cut was much higher in 2009 than in 2010: for example, yields of grasswhite clover were 5-3 and 2-4 t DM ha-1 and birdsfoot trefoil was 4.8 and 2.6 t DM ha-1, in 2009 and 2010 respectively.

Ash and cell contents

Large differences were found for ash and CP contents between harvests (P < 0.001, Table 1). Ash concentrations were lower in cuts 1 and 2 than in cuts 3 and 4. and increased from the second cut onwards, whereas CP concentrations were higher in the first than in the second cut and then increased to become highest in cuts 3 and 4 (data not shown).

The relations between N content and DM yield of the forage are illustrated for the non-leguminous forbs and legumes (Figure 2). There was no effect of slurry application on the relationship between these parameters. The non-leguminous forb species showed a nonlinear relationship and the legumes and the grasswhite clover mixture a linear negative relationship between yield and N concentration; this applied to

tein (CP), other compounds and ash, cell wall composition and in vitro organic matter digestibility (IVOMD) for eight species (four non-leguminous forbs, three forage legumes and a perennial ryegrass/white clover mixture), averaged over four cuts in 2009 and 2010. Table 1 Yield, dry-matter (DM) content and concentrations (g kg DM⁻¹) of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude pro-

	Salad			Ribwort	Yellow		Birdsfoot			S	Sign.	
Trait	burnet	Caraway	Chicory	plantain	sweet clover	Lucerne	trefoil	Mixture	Spec	Cut	SXC	Year
DMY (kg DM ha ⁻¹ year ⁻¹)	463647	5560 ^{ab}	3q0966	8416abc	3904ª	15 412 ^d	9460abc	12 548 cd	*	* *	SN	N
DM content (g DM kg ⁻¹)	182	160	116	170	169	184	143	145	SN	*	*	ON
DM composition (g kg DM ⁻¹)									2			CAT
NDF	295a	322 ^{ab}	329 ^b	402°	334 ^{bc}	383dc	328 ^{ab}	362 cd	e r	4 京水	V	No
ADF	240^{a}	270^{a}	275ª	339 ^b	271 ^a	321 ^b	272ª	275ª	* *	***	SN	SN
ADL	44ª	51 ^a	42ª	78 ^b	45ª	67 ^b	62 ^b	49ª	* *	*	SN	*
CP	132^{bc}	135°	102ª	115ab	198 ^d	200 ^d	206 ^d	p661	***	* *	*	N
Other‡	452^{f}	400^{de}	406°	362cd	341 abc	306ª	349bc	320ab	***	* *	SN	SN
Ash	62 _{ap}	118°	143 ^d	104^{ab}	108 ^{bc}	95 ^{ab}	91a	de/6	**	* *	*	SN
OM quality												0
Cell wall composition												
Cellulose§ (%)	99	89	70	65	89	99	63	19	SN	N	N	No
Hemicellulose¶ (%)	19	16	17	15	18	17	81	36	SN	***	* *	CNI
Lignin†† (%)	I Sabc	16 ^{bc}	13ª	p61	13 ^{ab}	17 cd	19 ^d	13 ^{ab}	*	* *	NC	*
IVOMD‡‡ (g kg OM^{-1})	641ª	743 ^{ef}	715 ^{de}	636a	703 cd	9e099	949L9	749 ^f	* *	*	SN	*
NS, not significant. Significance of main effects of species (Spec) and cut, their interaction (S × C) and of year (Y): ***, P < 0.001; **, P < 0.01; *, P < 0.05. †Within a row, least	ce of main	effects of specie	NS, not significant. Significance of main effects of species (Spec) and cut, their interaction (S $ imes$ (cut, their int	eraction ($S \times C$) ar	ıd of year (Y):	***, P < 0.00	l; **, P < 0.01	; *, P < 0	.05. *Wit	hin a row	least .

square means without a common superscript are significantly different (P < 0.05). Other = 100 - (NDF + CP + Ash) - mainly WSC and other components. \$100 × (ADF − ADL)/NDF. ¶100 × (NDF − ADF)/NDF. ^{+†}100 × ADL/NDF. ^{+†}1*n vitro* organic matter digestibility.

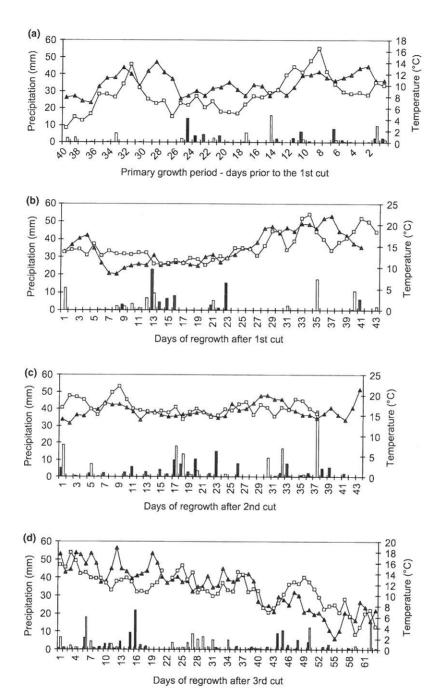


Figure I Rainfall and average air temperature in 2009 (black) and 2010 (open symbols) during (a) primary growth: 19 April–28 May 2009 (40 d) and 21 April–30 May 2010 (40 d); (b) first regrowth¹: 29 May–8 July 2009 (41 d) and 31 May–12 July 2010 (43 d); (c) second regrowth²: 9 July–21 August 2009 (43 d) and 13 July–19 August 2010 (37 d); (d) third regrowth: 21 August–22 October 2009 (63 d) and 19 August–20 October 2010 (63 d). Note the different temperature scales. ¹Irrigation was applied in 2009 during first regrowth on days 12 (9 June, 38 mm) and 32 (29 June, 54 mm) (not shown). ²Irrigation was applied in 2010 during second regrowth on day 3 (15 July, 15 mm) (not shown).

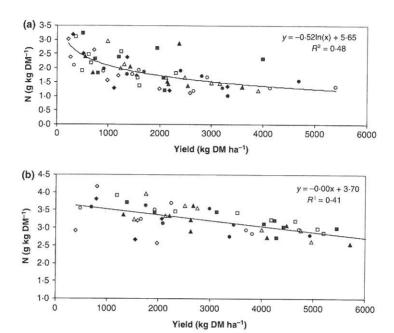


Figure 2 Relationships (P < 0.001) between nitrogen concentration and yield for (a) four non-leguminous forb species (◊ salad burnet, \Box caraway, \circ chicory, Δ ribwort plantain). (b) three legume species and a perennial ryegrass/white clover mixture (\Diamond yellow sweet clover, \Box luceme, \circ birdsfoot trefoil, Δ grass/clover). Open symbols depict unfertilized plots, filled symbols plots with slurry.

individual species (data not shown) as well as the overall group.

There were large differences (P < 0.001) between harvests in the concentration of 'other compounds' (Table 1) including water-soluble carbohydrates, which were highest in the first cut and lowest in the third cut (not shown).

Cell wall constituents and in vitro organic matter digestibility

The fibre contents (i.e. concentrations of NDF, ADF and ADL) differed between harvests (P < 0.001) and were lowest in the fourth harvest for all species. The concentrations of ADF and its components (hemicellulose and lignin) were higher in 2010 than in 2009 (P < 0.05, Table 1). No other effects of year were found. IVOMD differed between all four harvests (P < 0.001, Table 1) and increased from the second cut onwards. The highest IVOMD was in herbage at the fourth cut, followed by cuts 1, 3 and 2 respectively. IVOMD was on average lower in 2010 (P < 0.05, Table 1) and also more variable between species, and within most species, than in 2009. Despite variation in temperature during the third regrowth period (Figure 1d), IVOMD values of the fourth cut

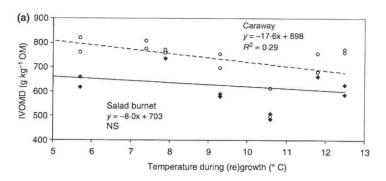
were comparable between years (Figure 3). There was no relation between yield and digestibility.

Relation between in vitro organic matter digestibility and temperature

Regression analyses showed a strong negative relation between IVOMD and temperature during regrowth (Figure 3) for each species. Although there were similar relationships for average, maximum and minimum temperatures during regrowth, the statistical significance was strongest for the latter.

The similarity in pattern and difference in level are shown in Figure 3a for salad burnet and caraway, and in Figure 3b, for chicory and ribwort plantain. For each species, the values of replicate plots can be seen across the temperature range. In general, replicate plots had similar values.

The slopes of the regression lines for the nonleguminous forbs were highest for chicory (-25.5), followed by ribwort plantain $(-22\cdot0)$, caraway (-17.6) and salad burnet (-8.0) (Figure 3). For the legumes, the slopes were yellow sweet clover: -14·1, lucerne: -16.4, birdsfoot trefoil: -18.5, and for grasswhite clover: -18.8; respective R^2 values 0.74, 0.55, 0.58 and 0.68 (P < 0.001, not shown).



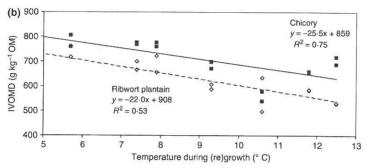


Figure 3 Relation between in vitro organic matter digestibility (IVOMD, g 100 g OM-1) and average daily temperature during (re)growth for (a) salad burnet (\blacklozenge , P = 0.28, not significant) and caraway (0, P < 0.05) and (b) chicory (\blacksquare , P < 0.01) and ribwort plantain (0, P < 0.001). Points on the X-axes reflect from left to right: cut 1 in 2010 and 2009, cut 4 in 2010 and 2009. cut 2 in 2009 and 2010, and cut 3 in 2010 and 2009.

Relation between in vitro organic matter digestibility and fibre

The negative relationships between IVOMD and NDF are illustrated for the non-leguminous forb and legume species in Figure 4. To avoid bias due to different ash contents among species (Table 1), NDF concentrations in organic matter (OM) are shown. Salad burnet had the narrowest range in NDF concentrations and a lower IVOMD than other non-leguminous 6 forbs at the same NDF level (Figure 5a).

Birdsfoot trefoil had a lower IVOMD than other legumes at the same NDF level, and the grass-white clover mixture had the highest IVOMD (Figure 4b). The relatively low R^2 of the regression line for the grass-white clover mixture, compared with pure stands of non-leguminous forbs and legumes, reflects the variability in proportions of grass and clover during the various harvests. The slopes of the regression lines were more negative for non-leguminous forbs, in particular salad burnet, than for most legumes and grass-white clover. This indicates that IVOMD declined most rapidly with higher NDF concentrations in salad burnet, while in yellow sweet clover, IVOMD was least responsive to changes in NDF. The IVOMD

level was highest (P < 0.001) in the grass–white clover mixture across the measured NDF range.

Discussion

Herbage yield and nitrogen uptake

Variation in growth pattern may be due to interactions with weather conditions that produced different effects in the 2 years. Growth in spring 2010 started late due to the cold winter and low April temperatures, and the effective primary growth period was shorter. This implies that the forage was less mature on 31 May 2010 than on 29 May 2009. Higher DM yield at the first cut in 2009 than in 2010 can also be related to higher radiation in 2009 (742 vs. $635 \text{ MJ} \text{ m}^{-2} \text{ during}$ the 40 d preceding the first cut). In addition, the leaf/ stem ratio may have been lower in 2009 due to extreme April drought (0 mm rainfall), which may have caused early maturation, although leaf/stem proportions were not determined. Yield fluctuations in cuts 2-4 would have been affected not only by the weather but also by the yield level of the previous cut, as heavy cuts delay the start of the next re-

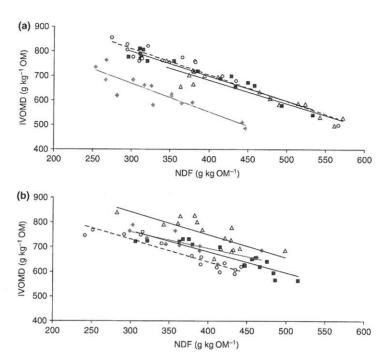


Figure 4 Relationships between IVOMD and NDF for (a) four non-leguminous forbs (* salad burnet, ■ chicory, ο caraway, Δ ribwort plantain; all P < 0.001). Grey symbols depict salad burnet, the dotted line depicts caraway. Respective R^2 of regression lines: 0.82, 0.94, 0.92 and 0.90; slopes: -1.15, -1.01, -1.07 and -0.95; (b) three legumes and a perennial ryegrass-white clover mixture (\spadesuit yellow sweet clover, P < 0.05, \blacksquare lucerne, P < 0.001, \circ birdsfoot trefoil, P < 0.001, Δ grass-clover, P < 0.01). Grey symbols depict yellow sweet clover, the dotted line depicts birdsfoot trefoil. Respective R2 of regression lines; 0.56, 0.83, 0.92 and 0.50; slopes: -0.58, -0.86, -1.04 and -1.05

As expected, lucerne and the grass-white clover mixture had greater DM yields than most of the tested non-leguminous forb species, although the DM yield of chicory, plantain and birdsfoot trefoil was similar to the grass-white clover mixture, confirming our hypothesis that some forb species can provide yields equal that of grass-clover. In this experiment, we investigated species sown as pure stands, and the yield of salad burnet ranked lowest. This finding contrasts with the high relative performance of this species in a sown mixture in work reported by Fisher et al. (1996), who found, in extensively managed swards of forbs sown singly with a standard grass mixture, that ribwort plantain, salad burnet, birdsfoot trefoil and chicory were among the species that competed well with grasses and produced annual forb-herbage yields greater than 2 t DM ha-1. This may have been due, at least in part, to the absence of fertilization in the 3-year experiment of Fisher et al. (1996), although plants can also interact differently with each other when grown in a mixture than when grown in monoculture (Casler et al., 1987).

At a site in Germany, ca. 250 km to the south of our experiment, Loges (2012) found that first-year yields (in 2010) of pure stands of forbs ranged from 3-3 t DM ha⁻¹ in salad burnet to 9-3 t DM ha⁻¹ in lucerne; the yields from chicory, birdsfoot trefoil and ribwort plantain were 6.7, 5.7 and 5.3 t DM ha⁻¹, respectively, and yellow sweet clover and caraway were 4.5 and 4.3 t DM ha⁻¹. In our experiment, firstyear yields (in 2009) were much higher for lucerne, chicory, birdsfoot trefoil and ribwort plantain (16.2, 13.0, 10.9 and 9.4 t DM ha⁻¹ respectively) possibly due to the earlier start of the growing season in 2009. The ranking order of these four species was similar; salad burnet yielded slightly more and caraway slightly less in our experiment (4.8 and 3.8 t DM harespectively). Caution should be taken when interpolating yield data. In this experiment, year effect was confounded with sward age (2009 was the first and 2010 the second year after establishment) which may have affected tiller density and (tap-) root develop-

Surprisingly, yields were similar in slurry-fertilized and mineral-K fertilized replicates, as were N

55

contents and quality parameters in the harvested herbage. In this experiment, carried out on highfertility agricultural soils, amounts of available N, P and K were apparently not limiting. The N uptake of non-leguminous forbs (yield x N content, Figure 2) on plots without slurry provided a basis for estimation of the N-delivery capacity of the experimental site, attributed to mineralization and N deposition (deposition was ca. 15 kg N ha⁻¹ y⁻¹ in Denmark at the time of the experiment). The N-delivery capacity was at least 200 kg N ha-1 in 2009 and 140 kg N ha⁻¹ in 2010, approaching the amounts of total N provided in slurry (185 and 195 kg N ha⁻¹ of which 60% was NH₄-N). The loss of slurry N due to ammonia volatilization may explain the absence of a response to the additional N provided in slurry.

Crude protein, fibre and 'other compounds'

Our data showed that CP concentrations were similar in lucerne and birdsfoot trefoil, and NDF concentrations were similar in chicory and birdsfoot trefoil (Table 1). This is in contrast to the results of Chapman et al. (2009) in Canada; they reported that lucerne had higher yields and CP concentrations than chicory and birdsfoot trefoil, and chicory had lower NDF concentrations than the other forages. In a study in the Netherlands, Warner et al. (2010) found higher concentrations of CP from June to August in ribwort plantain (87–115 g kg DM⁻¹) than in chicory $(101-123 \text{ g kg DM}^{-1})$, similar to our findings (Table 1), and a higher NDF content in ribwort plantain $(174-295 \text{ g kg DM}^{-1})$ than in chicory (169-174 g kg DM⁻¹), values that were much lower than in our experiment. Søegaard et al. (2011) also found higher NDF contents in ribwort plantain (338 and 466 g kg DM⁻¹ in cuts 1 and 3) than in chicory (235 and 352 g kg DM⁻¹ respectively). These values were comparable with ours (Table 1).

The 'other compounds' in salad burnet, chicory, caraway and ribwort plantain were 0.51, 0.47, 0.46 and 0.40 of OM respectively. The proportions were lower in yellow sweet clover and birdsfoot trefoil (0.38), in grass-white clover (0.35) and lucerne (0.34). These values are, however, prone to large errors because 'other compounds' were calculated as a difference from total, minus CP, NDF and FA concentrations. 'Other compounds' such as condensed tannins (e.g. Piluzza et al., 2014), saponins (e.g. Francis et al., 2002) and phenols (e.g. Loges, 2012) may play a role in animal nutrition and animal products; more research is needed to identify the various compounds in forbs and their functions.

Ash

Very high concentrations of ash (>150 g kg ha⁻¹) were found both in the species of Boraginaceae (borage and viper's bugloss), both of which have cuticular hairs. Although these values appear greater than for other species (Appendix 1), these results are not replicated and were not part of the statistical analysis. The grass-white clover mixture had similar ash contents to the legumes and non-leguminous forbs other than caraway and chicory (Table 1).

Warner et al. (2010) found higher ash concentrations in chicory (127-152 g kg ha⁻¹) than in ribwort plantain (102-115 g kg ha⁻¹) between May and August. The reported values on ash contents, as well as seasonal trends, are comparable with our findings.

Herbage digestibility, organic matter quality and intake

In general, there were large and consistent differences in nutritive value among species. The IVOMD values obtained in our study with sown single-species swards can be compared with the outcomes of a study of Søegaard et al. (2011) that investigated mixed swards at the same site, also under a 4-cut system and averaged over 0 N and 200 N slurry application treatments. The results of Søegaard et al. (2011) and this study showed that in spring, chicory had an IVOMD level comparable with grass, but a relatively large decrease towards summer, and an even greater decline was found in plantain. Plantain had a lower IVOMD than chicory. In contrast with other species, caraway maintained its high IVOMD from spring to summer. Birdfoot trefoil had IVOMD levels comparable with lucerne, in spring and in summer. The IVOMD of salad burnet was very low, as in Søegaard et al. (2011).

A strong negative relation between IVOMD and mean air temperature during regrowth was observed for all species, which is in agreement with findings of Wilson and Ford (1971) for perennial ryegrass. In 2010, the transition from the vegetative to the generative stage was probably delayed during the cold spring and enhanced after the first cut due to the change in weather. In addition, temperatures during the days before harvest were about 5°C higher (Figure 2b) which may have caused a decline in the concentration of sugars. These factors might have contributed to the lower IVOMD in the second cut of 2010 than in 2009, particularly in caraway and chicory (Figure 4).

Apart from digestibility and energy content, the nutritive value of forage as a feed for livestock is determined by voluntary intake. No clear relationships are described for most species, but generally, intake is

55

related to DM digestibility, structural carbohydrate content and breakdown capacity in the rumen (Armstrong et al., 1986). Legume intake is generally higher than grass intake because legumes have lower cell wall contents, higher CP concentrations and faster rates of particle size reduction in the rumen, and faster OM 8 removal from the rumen (Rook et al., 2002). Wilman et al. (1997) observed high voluntary intake in some forb species despite a high NDF concentration, probably because tissues of dicotyledonous species are broken down more easily than grass tissues in the rumen. In our study, ambiguous relations between IVOMD and NDF content were found for the various non-leguminous forbs, for example, the higher IVOMD of caraway than of ribwort plantain and salad burnet could not be explained by differences in the degree of cell wall lignification or proportions of cellulose or hemicellulose. Further research is needed to find out whether the ambiguous results found for ribwort plantain may be due to low rates of in vitro NDF degradability.

Implications for farming practice and recommendations

The results of this experiment have shown that lucerne, chicory, ribwort plantain and birdsfoot trefoil, in single-species sown stands, had similar DM yield to a perennial ryegrass-white clover mixture. In addition, potentially useful attributes of forbs as forages included low NDF content of salad burnet, caraway, birdsfoot trefoil and chicory relative to a perennial ryegrass-white clover mixture. The adoption of forbs in practice requires a number of challenges to be overcome, including establishment and persistence in mixed swards under cutting/grazing.

Acknowledgments

The project was funded by the Danish 'Fund for organic farming'. Technical assistance of the staff at Foulumgaard Experimental Station is acknowledged. We thank Dr K. Kristensen for statistical advice. Financial support of A. Elgersma (EU-COST FA0802 Feed for Health grants for short-term scientific missions to Foulum) is gratefully acknowledged.

References

- ARMSTRONG R.H., COMMON T.G. and SMITH H.K. (1986) The voluntary intake and in vivo digestibility of herbage harvested from indigenous hill plant communities. Grass and Forage Science, 41, 53-60.
- CASLER M.D., COLLINS M. and REICH J.M. (1987) Location, year, maturity, and alfalfa competition effects

- on mineral element concentrations in smooth bromegrass. Agronomy Journal, 79, 774-778.
- CHAPMAN G.A., BORK E.W., DONKOR N.T. and HUDSON R.J. (2009) Performance and dietary preferences of white-tailed deer grazing chicory, birdsfoot trefoil or alfalfa in north central Alberta. Journal of Animal Physiology and Animal Nutrition, 93, 794-801.
- COCHRAN W.G. and Cox G.M. (1957) Experimental designs. New York: Wiley.
- COLLINS M. and McCoy J.E. (1997) Chicory productivity, forage quality, and response to nitrogen fertilization. Agronomy Journal, 89, 232-238.
- ELGERSMA A., SØEGAARD K. and JENSEN S.K. (2012) Vitamin and fatty acid contents in forage herbs. Abstract. EU COST - 1st Farly Stage Researcher Workshop Feed your knowledge! Healthy feed to healthy food. Workshop 7-8 June 2012, Barcelona, Spain. Book of abstracts ESR workshop Feed your knowledge, pp. 24.
- FISHER G.E.J., BAKER L.J. and TILEY G.E.D. (1996) Herbage production from swards containing a range of grass, forb and clover species and under extensive management. Grass and Forage Science, 51, 58-72.
- FOSTER L. (1988) Herbs in pastures development and research in Britain, 1850-1984. Biological Agriculture and Horticulture, 5, 97-133.
- Francis G., Kerem Z., Makkar H.P.S. and Becker K. (2002) The biological action of saponins in animal systems: a review. British Journal of Nutrition, 88, 587-
- GLASSEY C.B., CLARK C.E.F., ROACH C.G. and LEE J.M. (2013) Herbicide application and direct drilling improves establishment and yield of chicory and plantain. Grass and Forage Science, 68, 178-185.
- HANSEN B. (1989) Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agriculturae Scandinavica, 39, 113-118.
- HOPKINS A. and WILKINS R.J. (2006) Centenary review: temperate grassland: key developments in the last century and future perspectives. Journal of Agricultural Science, 144, 503-523.
- JEANGROS B. and THOMET P. (2004) Multi-functionality of grassland systems in Switzerland. Grassland Science in Europe, 9, 11-23.
- JENSEN S.K. (2008) Improved Bligh and Dyer extraction procedure. Lipid Technology, 20, 280-281.
- Kunelius H.T. and MACRAE K.B. (1999) Forage chicory persists in combination with cool-season grasses and legumes. Canadian Journal of Plant Sciences, 79,
- LABREVEUX M., HALL M.H. and SANDERSON M.A. (2004) Productivity of chicory and plantain cultivars under grazing. Agronomy Journal, 96, 710-716.
- LI G. and KEMP P.D. (2005) Forage chicory (Cichorium intybus L.): a review of its agronomy and animal production. Advances in Agronomy, 88, 187-222.
- LINDSTRÖM B.E.M., FRANKOW-LINDBERG B.E., DAHLIN A.S., WIVSTAD M. and WATSON C.A. (2013) Micronutrient concentrations in common and novel forage species and varieties grown on two contrasting soils. Grass and Forage Science, 68, 427-436.

- LITTELL R.C., MILLIKEN G.A., STROUP W.W. and WOLFINGER R.D. (2006) SAS_ systems for mixed models. Cary, NC, USA: SAS Institute.
- Loges R. (2012) Urter og tanninrige planter den nyeste tyske forskning. [Herbs and tannin-rich plants - the latest German research.] In: ???? ????. (ed.) Plantekongres 2012 - produktion, plan og miljø, pp. 363-365. Herning, Denmark: ????.
- MERTENS D.R. (2002) Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International, 85, 1217-1240.
- MICHAUD A., ANDUEZA D., PICARD F., PLANTUREUX S. and BAUMONT R. (2012) Seasonal dynamics of biomass production and herbage quality of three grasslands with contrasting functional compositions. Grass and Forage Science, 67, 64-76.
- MOLONEY A.P., FIEVEZ V., MARTIN B., NUTE G.R. and RICHARDSON R.I. (2008) Botanically diverse foragebased rations for cattle: implications for product composition, product quality and consumer health. Grassland Science in Europe, 13, 361-374.
- PILUZZA G., SULAS L. and BULLITTA S. (2014) Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. Grass and Forage Science, ????, ????-???? (in press, doi: 10.1111/gfs. 10 12053).
 - PIRHOFER-WALZL K., SØEGAARD K., HØGH-JENSEN H., ERIKSEN J., SANDERSON M.A., RASMUSSEN J. and RASMUSSEN J. (2011) Forage herbs improve mineral composition of grassland herbage. Grass and Forage Science, 66, 415-423.
 - ROOK A.J., HARVEY A., PARSONS A.J., PENNING P.D. and ORR R.J. (2002) Effect of long-term changes in relative resource availability on dietary preference of grazing sheep for perennial ryegrass and white clover. Grass and Forage Science, 57, 54-60.
 - SANDERSON M.A., LABREVEUX M., HALL M.H. and ELWINGER G.F. (2003a) Forage yield and persistence of chicory and English Plantain. Crop Science, 43, 995-1000.
 - SANDERSON M.A., LABREVEUX M., HALL M.H. and ELWINGER G.F. (2003b) Nutritive value of chicory and English plantain. Crop Science, 43, 1797-1804.

SCHELLBERG J. and PONTES DA L.S. (2012) Plant functional traits and nutrient gradients on grassland. Grass and Forage Science, 67, 305-319.

M

12

- SMIDT N.W. and BRIMER L. (2005) The use of herbs in pastures: an interview survey among bio-dynamic and organic farmers with dairy cattle. Agricultural and Human Values, 22, 355-363.
- SØEGAARD K., ERIKSEN J. and ASKEGAARD M. (2011) Herbs in high producing organic grasslands - effect of management. In: Neuhoff D., Halberg N., Rasmussen I.A., Hermansen J., Ssekyewa C. and Sohn S.M. (eds) Proceedings of the Third Scientific Conference of ISOFAR, Vol. 1 Organic Crop Production, pp. 190-193. ????: ????.
- VAN SOEST P.J. (1963) Use of detergents in the analysis of fibrous feeds. Journal of the Association of Official Agricultural Chemists, 46, 825-835.
- SOIL SURVEY STAFF. (1998) Ultisols. In: SOIL SURVEY STAFF. (ed.) Keys to Soil Taxonomy, pp. 267-269. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service.
- TILLEY J.M.A. and TERRY R.A. (1963) A two-stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society, 18, 104-111.
- VOISIN A. (1959) Grass productivity. London: Crosby Lockwood.
- WARNER D., JENSEN S.K., CONE J.W. and ELGERSMA A. (2010) Fatty acid composition of forage herb species. Grassland Science in Europe, 15, 491-493.
- WILMAN D., DERRICK R.W. and Moseley G. (1997) Physical breakdown of chickweed, dandelion, dock, ribwort, spurrey and perennial ryegrass when eaten by sheep and when macerated. Journal of Agricultural Science, 129, 419-428.
- WILSON J.R. and FORD C.W. (1971) Temperature influences on the growth, digestibility, and carbohydrate compositions of two tropical grasses, Panicum maximum var Trichoglume and Setaria sphacelata, and two cultivars of the temperate grass Lolium perenne. Australian Journal of Agricultural Research, 22, 563-571
- Younie D. (2012) Grassland management for organic farmers, pp. 208. Marlborough, UK: The Crowood Press.

APPENDIX

Concentrations (g kg⁻¹ DM) of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude protein (CP), 'other compounds' and ash, and in vitro organic matter digestibility (IVOMD, g kg⁻¹ OM) of chervil (Anthriscus cerefolium L.), borage (Borago officinalis L.) and viper's bugloss (Echium vulgare L.).

	2009 Cut 1 Anthriscus cerefolium	2009 Cuts 2 + 3 Borago officinalis	2009 Cuts 2 + 3 Echium vulgare	2010 Cuts 1–4 Echium vulgare
NDF	412	369	316	365
ADF	298	359	307	350
ADL	62	94	67	106
CP	92	109	154*	145
Other	386	363	306*	312
Ash	80	151	203*	165
IVOMD	670	620	590*	610

^{*} Samples available from the third cut only.

Author Query Form

Journal:

GFS

Article:

12104

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication. Many thanks for your assistance.

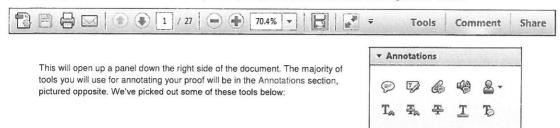
Query reference	Query	Remarks
1	AUTHOR: Please provide the organization name for the affiliation which has the link symbols '*'.	
2	AUTHOR: Please check that authors and their affiliations are correct.	
3	AUTHOR: Please check the address details for corresponding author.	
4	AUTHOR: Supplementary Material/Supporting Information has been cited in the text. Please forward the necessary files to the Production Editor so that they can appear online with your paper.	
5	AUTHOR: SAS Institute, 2001 has not been included in the Reference List, please supply full publication details.	
6	AUTHOR: Only citation has been provided for Figure 5a. Hence please provide a suitable legend and image for Figure 5.	
7	AUTHOR: Piluzza et al., 2013 has been changed to Piluzza et al., 2014 so that this citation matches the Reference List. Please confirm that this is correct.	
8	AUTHOR: Rook and Yarrow, 2002 has been changed to Rook <i>et al.</i> , 2002 so that this citation matches the Reference List. Please confirm that this is correct.	
9	AUTHOR: Please provide the editor name(s), publisher name for reference Loges (2012).	
10	WILEY: Please provide the volume number, page range for reference Piluzza et al. (2014).	
11	AUTHOR: Please check forename for author 'Pontes L. da S.' in reference Schellberg and Pontes (2012).	
12	AUTHOR: Please provide the publisher name, publisher location for reference Søegaard et al. (2011).	

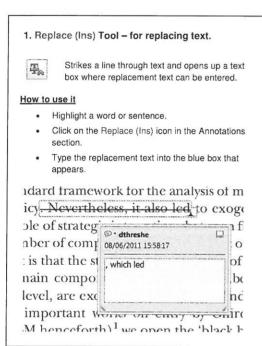
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

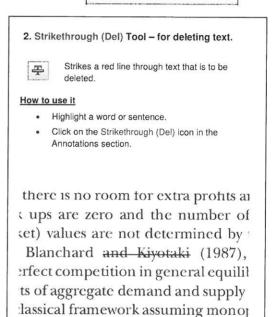
Required software to e-Annotate PDFs: <u>Adobe Acrobat Professional</u> or <u>Adobe Reader</u> (version 8.0 or above). (Note that this document uses screenshots from <u>Adobe Reader X</u>)

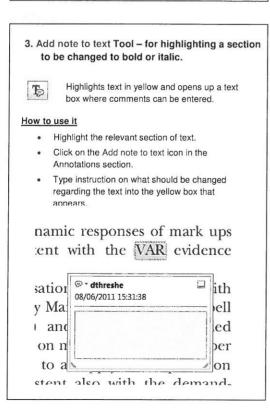
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/reader/

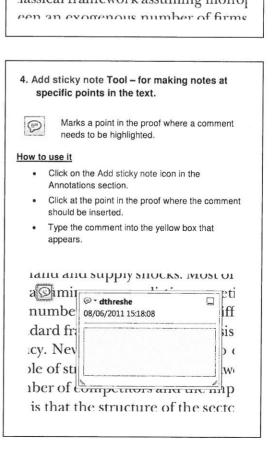
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:











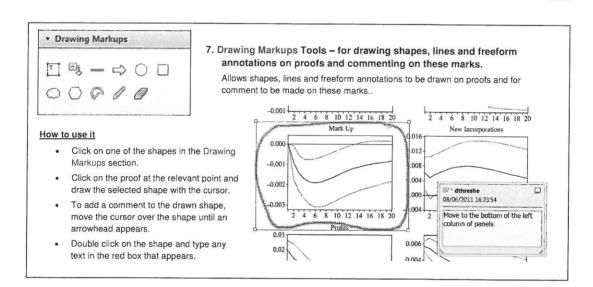


USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool - for inserting large amounts of text or replacement figures. Inserts an icon linking to the attached file in the 6 appropriate pace in the text. How to use it Click on the Attach File icon in the Annotations section. Click on the proof to where you'd like the attached file to be linked. Select the file to be attached from your computer or network. Select the colour and type of icon that will appear in the proof. Click OK. END 0.20 0.15 0.10

0.05

6. Add stamp Tool - for approving a proof if no corrections are required. Inserts a selected stamp onto an appropriate place in the proof. How to use it · Click on the Add stamp icon in the Annotations section. Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears). Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page). of the business cycle, starting with the on perfect competition, constant ret 21 otaki (1987), has introduced produc general equilibrium models with nomin and and a committee than the Mantage of the life time.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options:

