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Impact of maturation on extractable polyphenols in leguminous fodder species

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Abstract. Legume trees and shrubs are valuable feed sources for small ruminants although their antiquality factors. The aim of the present study was to investigate differences in total phenols (TPH), total tannins (TT) and condensed tannins (CT) content in leaves and stems of three legume ligneous species (*Amorpha fruticosa, Robinia pseudoacacia* and *Colutea arborescens*) at two different stages of maturity: (i) during the season of rapid growth (May) when the leaves were young, and (ii) in September (mature) when growth had ceased and the woody parts had been hardened. Samples were analysed for their TPH, TT and CT content. According to the results, *R. pseudoacacia* had significantly higher content of TPH, TT, CT in leaves in May, compared to the other two species. Inversely, *A. fruticosa* had significantly higher TPH and TT content in the stems, while *C. arborescens* had the lowest one in both leaves and stems for the same period. In September, there was no significant difference in the content of TPH and TT in the leaves among species, except for *C. arborescens* which had significantly lower concentration. From the tested species, *C. arborescens* had the lowest content of all extractable polyphenols, without significant differences to leaves and stems in May and September respectively.

Keywords. Condensed tannins – Phenological stage – Leaves – Phenols – Forage quality.

Impact de la maturité sur les polyphénols extractibles chez les espèces de légumineuses arborescentes

Résumé. *Les légumineuses arborescentes ou arbustives sont une source d'alimentation précieuse pour les petits ruminants, malgré certains facteurs antinutritionnels. Le but de cette étude était d'étudier les différences de concentrations en phénols totaux (TPH), tanins totaux (TT) et tanins condensés (CT) dans les feuilles et les tiges de trois espèces ligneuses de légumineuses à différents stades de maturité:* Amorpha fruticosa, Robinia pseudoacacia *et* Colutea arborescens*. Trois plantes de chaque espèce ont été sélectionnées au hasard à deux périodes distinctes (stades de maturité) : jeunes feuilles pendant la saison de croissance rapide (Mai) et début Septembre (maturité) alors que la croissance avait cessé et les parties ligneuses avaient durci. Les échantillons ont été analysés pour leurs teneurs en TPH, TT et CT. Selon les résultats,* R. pseudoacacia *avait des teneurs significativement plus élevées en TPH, TT, TC dans les feuilles en Mai, par rapport aux deux autres espèces. Inversement,* A. fruticosa *avait des teneurs nettement plus élevées de TPH et TT dans les tiges, tandis que* C. arborescens *avait la teneur la plus faible à la fois dans les feuilles et les tiges à cette même période. En Septembre, il n'y avait pas de différence significative des teneurs en TPH et TT dans des feuilles de* R. pseudoacacia *et* A. fruticosa*, à la différence de* C. arborescens *qui avait des concentrations significativement plus faibles. Des espèces testées,* C. arborescens *avait les teneurs les plus faibles en polyphénols extractibles, en Mai comme en Septembre et quel que soit la partie de la plante.*

Mots-clés. *Tanins condensés – Stade phénologique – Feuilles – Phénols – Qualité du fourrage.*

I – Introduction

Browse species can provide green forage for grazing animals throughout the year (evergreen species) or during critical periods (deciduous ones) (Kokten *et al.,* 2012). Despite their potential use as feed resources for ruminants and wild animals, most ligneous species contain anti-nutritive components such as phenolic compounds and especially tannins (Sallam *et al.,* 2010). Due to their nature and depending on their concentration, tannins have differential effects (Rana *et al.*, 2006) on animals ranging from beneficial to toxicity and even death (Makkar, 2003a).

Leaves contain more than 40% of the total nitrogen contained in a woody species (Kramer and Kozlowski, 1979) and their nutritive value is higher than the stems (Cordesse *et al.*, 1991). However, the intake of leaves from ligneous species by herbivores is often restricted by defending or deterring mechanisms related to high tannin content (Provenza, 1995). Maturity stage, leaf age and season (Rogler and Sell, 1984; Schultz *et al.,* 1982), are some of the factors affecting the concentration of tannins.

The objective of this research was to investigate the impact of maturity stage in the concentration of phenols, tannins, and condensed tannins separately in leaves and stems of three ligneous species.

II – Materials and methods

The experiment was carried out at the Aristotle University's farm (40° 34' E, 23° 43' N, at sea level) in northern Greece. The climate of the area could be characterized as Mediterranean semi-arid with cold winters. The mean annual temperature and precipitation are 15.5 °C and 443 mm, respectively.

Three broadleaved deciduous leguminous fodder species *Robinia pseudoacacia* var. monophylla (L.), *Amorpha fruticosa* L. and *Colutea arborescens* L. were investigated. For each species, samples were hand-plucked (i.e., leaves and twigs with diameter <3mm) from three individual plants at two discrete periods and stages of maturity: (i) during the season of rapid growth (immature: May, IM) when the leaves were young, and (ii) in September (mature: September, M) when growth had ceased and the woody parts had been hardened.

Upon collection, each plant was divided into leaves and stems and oven-dried at 50 °C for 48 h. All the samples were ground through a 1 mm sieve and were analysed for total phenols (TPH), total tannins (TT) and condensed tannins (CT) in three replicates according to Makkar (2003b). Total phenols (TPH) and total tannins (TT) in the extract were determined by a modification of the Folin-Ciocalteu method using polyvinylpolypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols. Both TPH and TT were expressed as tannic acid equivalent (mg/g TAE) (Makkar *et al*., 1993). Condensed tannins (CT) were determined according to the method of Porter *et al*. (1986), using purified quebracho CT as the reference standard and therefore expressed as quebracho equivalent.

Two-way ANOVA of the data was performed using SPSS® statistical software v. 18.0 (SPSS Inc., Chicago, IL, USA), in order to determine differences among the three species in the two stage of maturity in leaves and stems separately. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

In immature stage, *R. pseudoacacia* had higher concentration of TPH, TT and CT in the leaves compared to that of the other species, followed by *A. fruticosa*. *C. arborescens* had the lowest concentration in all measured components. In mature stage, *R. pseudoacacia* had higher CT concentration than the other species, while *R. pseudoacacia* and *A. fruticosa* had significant higher TPH and TT concentration in the leaves than *C. arborescens*. There was a significant interaction between season x species for TPH TT and CT (Table 1). The TPH and TT concentration in leaves of *R. pseudoacacia* was significantly higher in immature stage than in mature, while stage of maturity did affect the concentration of other two species. Moreover, maturity significantly reduced the CT concentration in leaves of *R. pseudoacacia* and *A. fruticosa,* but did not affected *C. arborescens*.

Table 1. TPH (mg/g DM TAE), TT (mg/g DM TAE), CT (mg/g DM QE) concentration of leaves from leguminous browse plants at immature (IM) and mature stage (M)

Species/Leaves	TPH (mg/g d.w. T.A.E)		TT (mg/g d.w. T.A.E)		CT (mg/g d.w. Q.E)	
	(IM)	(M)	(IM)	(M)	(IM)	(M)
Robinia pseudoacacia	26.5a†	20.1b	26.0a	20.0b	101a	82.5b
Amorpha fruticosa	16.5c	18.3bc	16.2.b	18.0b	68.0c	40.0d
Colutea arborescens	7.3d	7.6d	7.0c	7.2c	1.3e	1.0e

† Means for the same component with different letters are significantly different ($P \leq 0.05$).

Concerning the stems, *A. fruticosa* had the highest concentration of TPH, TT compared to the other two species in both stage of maturity. However, *A. fruticosa* had significantly higher CT concentration than the other species in mature stage, but only than *C. arborescens* in immature stage (Table 2). There was a significant interaction between season x species for TPH, TT and CT concentration of stems. Both *R. pseudoacacia* and *A. fruticosa decreased significant* the TPH and TT concentration from immature to mature stage, but not *C. arborescens.* The CT concentration significantly decreased with maturity only of *R. pseudoacacia.* Generally, leaves had higher concentration of TPH, TT and CT than stems in both maturity stages (Tables 1, 2). Similarly, Salawu *et al.* (1997) reported that the leguminous species *Calliandra calothyrsus* had more CT in leaves than in stems.

Table 2. TPH (mg/g DM TAE), TT (mg/g DM TAE), CT (mg/g DM QE) concentration of stems from leguminous browse plants at immature (IM) and mature stage (M)

Species/Leaves	TPH (mg/g d.w. T.A.E)		TT (mg/g d.w. T.A.E)		CT (mg/g d.w. Q.E)	
	(IM)	(M)	(IM)	(M)	(IM)	(M)
Robinia pseudoacacia	5.7c†	3.8d	5.6c	3.8d	17.0a	9.2b
Amorpha fruticosa	9.7a	7.7b	9.4a	7.4b	16.3a	19.5a
Colutea arborescens	2.6d	4.5cd	2.4e	4.4cd	0.4c	0.4c

† Means for the same component with different letters are significantly different ($P \leq 0.05$).

Contrasting results have been reported regarding the concentration of phenols in relation to the maturity stage. Glyphis and Puttick (1988) found that the concentration of phenols increased or remained stable as leaves became more mature. In contrast, Waterman and McKey (1989) reported that leaves had higher concentration of phenols in spring than in autumn. The results of the present study provide evidence than the concentration of phenols through maturity is species depended. There is a decrease in leaves CT concentration from spring to autumn for *R. pseudoacacia* and *A. fruticosa*. Several factors may affect the concentration and the solubility of CT in leaves. The quantity of CT to the foliage could vary according to the genotype (Baldwin *et al.,* 1987) and their concentration and extractability changes with season (Hagerman, 1998; Salem, 2005).

IV – Conclusions

R. pseudoacacia had the highest concentration among the tested species for all the measured components in leaves in spring and for CT in autumn, while *C. arborescens* the lowest ones. Concentrations in stems were generally lower compared to these in the leaves. The results of the present study indicate that the impact of maturation on the type and the concentration of phenols and tannins is species depended.

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