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Identification of six Papilionaceae species by epidermal characteristics: Microanalysis of hand-composed mixtures

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Summary: The microhistological analysis quantifies the botanical composition of herbivore diets by the identification of the epidermal characters of ingested species. Many Papilionaceae form part of herbivore diets due to their high nutritive quality. Different species of this family are sometimes grouped together due to the difficulty to recognize them. In this study, the epidermis of *Lotus tenuis*, *Medicago arabica*, *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense* and *Trifolium repens* was analysed. Also, it was tested if such descriptions could be useful to identify fragments of these species in hand-composed mixtures and then quantified by microanalysis. Descriptions and drawings are presented. Regressions were significant ($p<0.05$) for *Lotus tenuis*, *Medicago arabica*, *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense*, and not significant ($p>0.05$) for *Trifolium repens*. The slopes of the significant regressions did not differ from 1 ($p>0.05$). The identification of these species when present in herbivore faeces or in digestive tract contents is possible.

Key words: epidermis, microhistological analysis, herbivore diet, Fabaceae, Leguminosae, *Lotus*, *Medicago*, *Melilotus*, *Trifolium*.

Resumen: Identificación de seis especies de Papilionaceae mediante características epidérmicas: microanálisis de mezclas compuestas a mano. El análisis microhistológico cuantifica la composición botánica de la dieta de herbívoros mediante la identificación de los caracteres epidérmicos de las especies ingeridas. Dado su alto valor nutricional, muchas Papilionaceae forman parte de la dieta de los herbívoros. Diferentes especies de esta familia son a veces agrupadas debido a la dificultad para su reconocimiento. En este trabajo se analizaron las epidermis de *Lotus tenuis*, *Medicago arabica*, *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense* y *Trifolium repens*. También, se testeó si dichas descripciones podrían ser útiles para identificar fragmentos de estas especies en mezclas compuestas a mano y luego cuantificadas por microanálisis. Se presentan descripciones y dibujos. Las regresiones resultaron significativas ($p<0.05$) para *Lotus tenuis*, *Medicago arabica*, *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense*, y no significativa ($p>0.05$) para *Trifolium repens*. Las pendientes de las regresiones significativas no difirieron de 1 ($p>0.05$). La identificación de estas especies cuando están presentes en las heces o en contenidos del tracto digestivo de los herbívoros es posible.

Palabras clave: epidermis, análisis microhistológico, dieta herbívoros, Fabaceae, Leguminosae, *Lotus*, *Medicago*, *Melilotus*, *Trifolium*.

Introduction

In studies of botanical composition of herbivore diets utilizing microanalysis techniques (Sparks & Malecheck, 1968), different species of the family Papilionaceae are sometimes grouped together due to the difficulty to recognize them (Comparatore *et*

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al., 2001). But this difficulty depends on which legumes are present together in the field and in the diet. These species have high quality and digestibility, and are commonly found in herbivore diets (Camezzana, 1987; Bonti *et al*., 1995; Martella *et al*., 1996; Pelliza *et al.,* 1997). In studies of the dietary habits of *Rhea americana albescens* (Common Rhea) in grasslands of Buenos Aires Province, Argentina, Isacch *et al*. (2001) found a high percentage of *Medicago* sp., and Vacarezza (2001) was able to identify three different legumes.

There are few descriptions of the epidermal characteristics of legumes present in grasslands of Buenos Aires Province, Argentina (Arambarri & Colares, 1993; Manganaro, 1923; Stenglein *et al*., 2003; Yagueddú & Cid, 1992). Besides, there are no comparisons among different legumes that allow their recognition in mixtures. Therefore, in this study, the epidermis of *Lotus tenuis*, *Medicago arabica*, *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense* and *Trifolium repens* was analysed. Also, it was tested if such descriptions could be useful to identify fragments of these species in hand-composed mixtures. It is appropriate to make clear that these are cosmopolite plants. Besides they are cultivated in pastures.

Materials and Methods

Plants of *Lotus tenuis* Waldst. & Kit, *Medicago arabica* (L.) Huds., *Medicago lupulina* L., *Melilotus albus* Medik, *Trifolium pratense* L. and *Trifolium repens* L., were collected from three differents sites in grasslands of Balcarce, Buenos Aires Province, Argentina. Fourty fully expanded young and mature leaves per species were selected and diafanized using the technique of Dizeo de Strittmater (1973) and mounted in gelatine-glycerine. The following epidermal characteristics of adaxial and abaxial surface of the leaflets were analysed using all histologycal slides: type of trichomes and stomata complex, form of the epidermal cells and cell wall patterns. Length and width (µm) of epidermal cells, and length, width and density (number/mm^2) of stomata and trichomes, were estimated based on ten random measurements per species with a Leica ATC 2000 light microscope equipped with an ocular micrometer. Qualitative microcharacters and measurements are presented in tables.

Original drawings of the epidermal tissue were carried out with an Olympus CH3 light microscope equipped with a camera lucida. Besides, to test if the epidermal characteristics described for each species could be useful to identify them, 15 mixtures were hand composed with dried and ground material. Each mixture had a different combination and proportion of three of the studied species. They were cleared in 50% sodium hypochlorite (NaOCl) for 5 to 10 min. When the material turned yellowish, three washes were carried out in distilled water to remove the sodium hypochlorite. The transparent mixture was mounted in gelatine-glycerine. The percentage of each species in the mixture was quantified by the microanalysis technique (Sparks & Malechek, 1968). Regression analyses were performed for each species with these data and the real percentage of the species in the mixtures. Graphs are presented. By means of a Student's *t* test, Ho: B=1 was tested. STATISTICA 5.5 Program was used.

Results

There were no differences between young and mature leaves (Fig. 1, 2). The epidermal cells were, in general, isodiametric. But *Medicago arabica*, *Trifolium pratense* and *Trifolium repens* also presented elongated to isodiametric epidermal cell form (Table 1). The anticlinal cell walls of all studied species were curved and undulated U- or V-shaped on the abaxial surface, and straight and straight to curved in the adaxial one (Table 1).

The outside periclinal surface of each epidermal cell in all of the six studied species was convex and sometimes beared papillae (Fig. 1, 2). When present, papillae in *Medicago arabica*, *Medicago lupulina*, *Melilotus albus* and *Trifolium pratense*, were in both epidermal surfaces. Sometimes, papillae were in leaflet margins and in the costal zones. Whereas, in *Lotus tenuis* and *Trifolium repens*, papillae were observed in the adaxial epidermis.

The leaves of all species were amphistomatic and had anisocytic and anomocytic types of stomatal

apparatus (Fig. 1, 2). *Lotus tenuis*, *Medicago lupulina*, *Melilotus albus* and *Trifolium pratense* presented sunken stomata in adaxial and abaxial epidermis. *Medicago arabica* and *Trifolium repens* had sunken stomata in adaxial epidermis, but in the abaxial one the latter presented stomata over the level of the other epidermal cells and the former had both stomata positions in the same leaflet. The sunken stomata in all the species were surrounded by three or four epidermal cells, which form triangular or trapezoidal spaces over them (Fig. 1, 2). Stomata length varied from 15 to 30 μm and the width from 12.5 to 22.5 μm in all the species. Stomata density (number/mm^2) ranged from 152.8 to 407.6 in the adaxial epidermis and from 76.4 to 407.6 in the abaxial one. Whereas *Medicago arabica* and *Trifolium pratense* had similar stomatal density in the adaxial and abaxial epidermis, the rest of the species presented a higher density on the adaxial one (Table 2).

Two types of trichomes were found: eglandular and glandular. Among the eglandular trichomes two types were observed: the typical uniseriated trichome and a multicellular uniseriated trichome (the latter only in *Medicago arabica)*. All studied species presented the typical uniseriated trichomes of the Papilionaceae with different density and size of the cells that compose it (Table 3). These trichomes, in general, present a couple of short proximal cells and one long distal cell, except in *Trifolium pratense* which presents only one short proximal cell (Fig 2: B). This latter characteristic was observed, sometimes, in the typical uniseriated trichomes of *Trifolium repens*. *Lotus tenuis* and *Trifolium repens* presented these eglandular trichomes in both epidermis grouped in the petiolules and, besides, the former species presented them at the base of the leaflets. In *Medicago arabica* and *Melilotus albus* these trichomes were absent in the adaxial epidermis.

Trifolium pratense presented the longest typical uniseriated trichomes (635 to 1357.5 μm) while in the other species the total length oscillated from 195 to 707.5 μm (Table 3). Also, *Trifolium pratense* presented the widest proximal short cell (from 45 to 62.5 μm) while the remainder species presented values from 7.5 to 30 μm (Table 3). The highest trichome density was found in *Medicago lupulina* in the abaxial epidermis (from 19 to 44.5 trichomes/mm^2), and was 33.5 to 57% lower in the adaxial one (Table 3). Besides the typical uniseriated trichomes, *Medicago arabica* presented scarce and large eglandular uniseriated trichomes (from 460 to 930 μm length) constituted by a variable number of cells (from 12 to 18) with thin walls, and located at the base of the leaflets and in the petiolules (Fig. 1: B).

In regard to glandular trichomes, the ones of *Medicago arabica* and *Medicago lupulina* are formed by one short proximal cell, a foot constituted by one cell aproximately twice longer than the proximal cell, and a head with several cells. The head of the glandular trichomes of *Medicago arabica* has five to seven cells, some subapical arranged in a form biseriate (Fig. 1: Bf). But the head of the ones of *Medicago lupulina* has three to four cells. When there are three, the apical ones are arranged in a biseriate form, and when there are four they are disposed in a biseriate form (Fig. 1: Cf).

Melilotus albus glandular trichomes present one short proximal cell, a foot formed by two slightly elongated cells so arranged uniseriate and a head formed by one to four cells generally disposed uniseriate (Fig. 2: Af). The ones of *Trifolium pratense* present a short proximal cell from isodiametric to slightly elongated, a foot one celled, sometimes two to four celled so arranged uniseriate, and a head with four cells disposed uniseriate (Fig. 2: Bf). The ones of *Trifolium repens* present one short proximal cell which continues with a slightly elongated cell foot and the head formed by four cells arranged uniseriate to six cells some of them arranged in a form biseriate (Fig. 2: Cf).

Lotus tenuis was the only species that lacked glandular trichomes and *Trifolium pratense* presented them only sometimes at the margin of the leaflets. *Medicago arabica*, *Melilotus albus* and *Trifolium repens* did not present glandular trichomes in the adaxial epidermis. In general, the density of glandular trichomes respect to the typical eglandular uniseriated ones was smaller and more variable. Glandular trichome characteristics can be seen in Figures 1 and 2, and Table 4.

Figure 3 shows the curves of the regression analysis of each of the studied species. Regressions were significant (p<0.05) for *Lotus tenuis* (n=8, p=0.000594), *Medicago arabica* (n=7, p=0.013327),

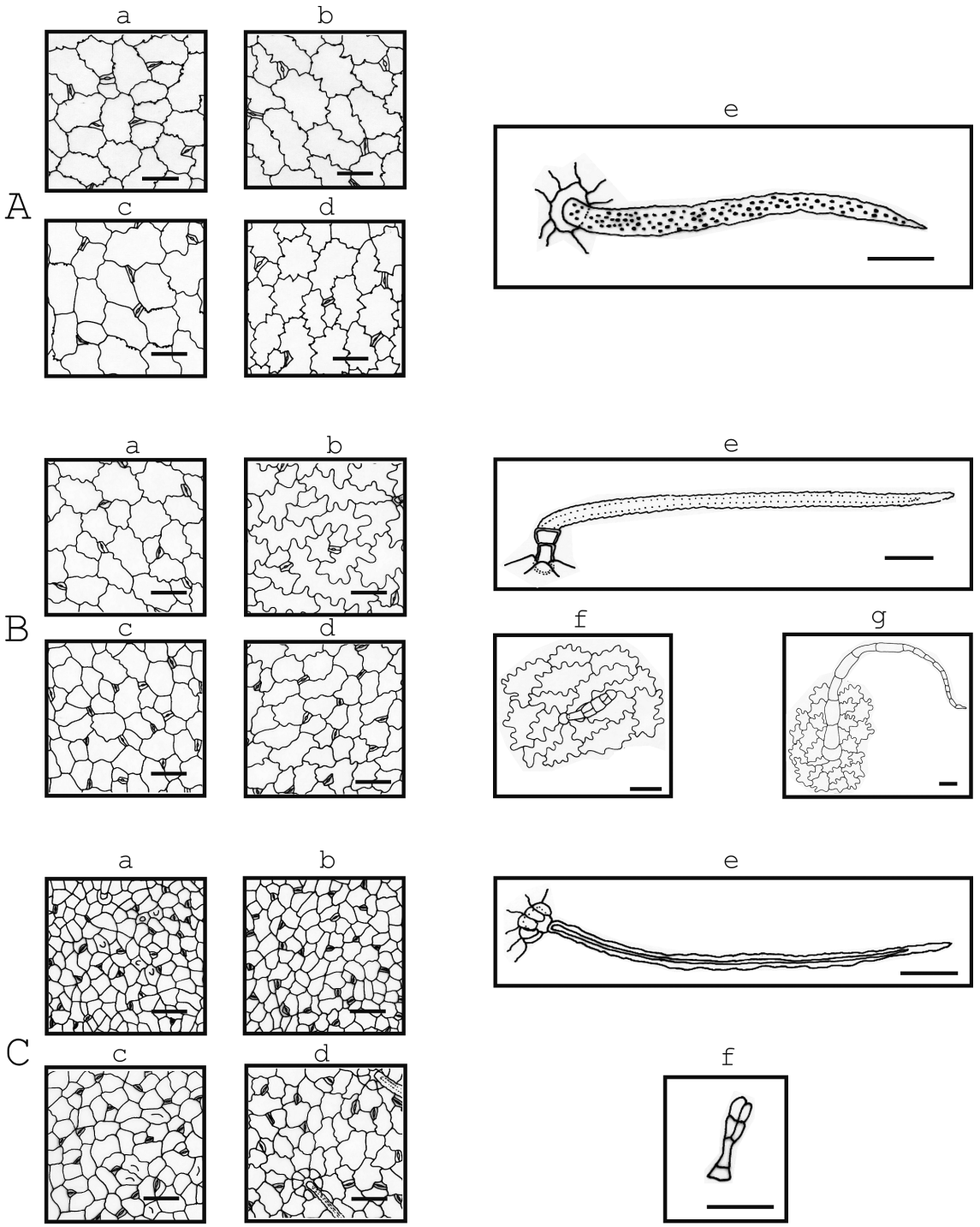

Fig. 1. Drawings of epidermal tissue of: A, *Lotus tenuis* Waldst. & Kit; B, *Medicago arabica* (L.) Huds.; C, *Medicago lupulina* L.; a & b, young leaflet; c & d, mature leaflet; a & c, adaxial epidermis; b & d, abaxial epidermis. Presenting papillae: C, a & c. Trichomes: e, typical uniseriated eglandular trichomes; f, glandular trichomes; g, uniseriated eglandular multicellular trichomes. Scale bar = 50 μm

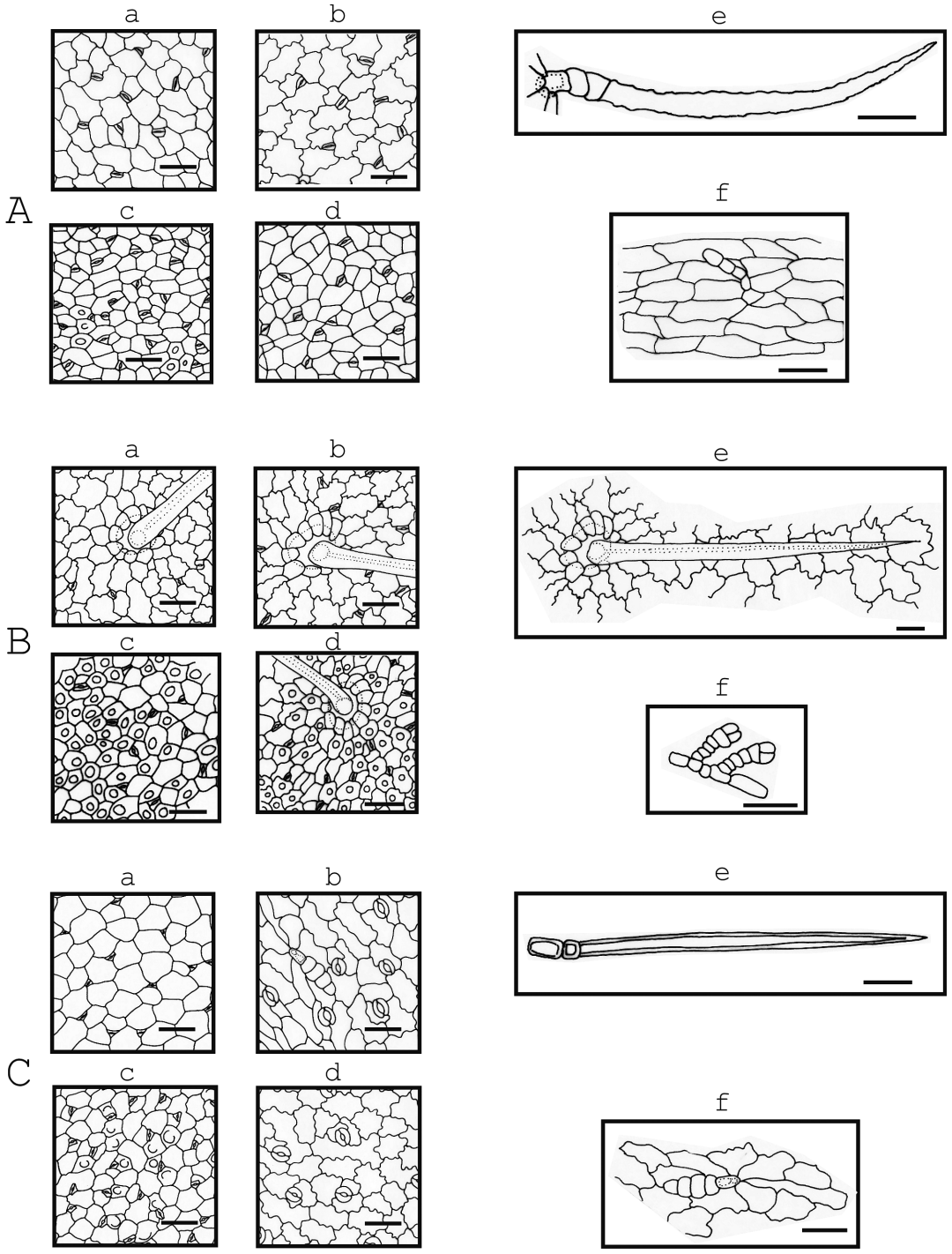

Fig. 2. Drawings of epidermal tissue of: A, *Melilotus albus* Medik; B, *Trifolium pratense* L.; C, *Trifolium repens* L. a & b, young leaflet; c & d, mature leaflet; a & c, adaxial epidermis; b & d, abaxial epidermis. Presenting papillae: A, c; B, c & d; C, c. Trichomes: e, typical uniseriated eglandular trichomes; f, glandular trichomes. Scale bar = 50 μm

Medicago lupulina (n=7, p=0.000040), *Melilotus albus* (n=7, p=0.043359), *Trifolium pratense* (n=8, p=0.000627), and not significant (p>0.05) for *Trifolium repens* (n=8, p=0.180552). The slope of the significant regressions did not differ from 1 (p>0.05), *Lotus tenuis* p=0.45592686, *Medicago arabica* p=0.4779881, *Medicago lupulina* p=0.650604637, *Melilotus albus* p=0.11459676 and *Trifolium pratense* p=0.071558494.

In the analysed mixtures, *Medicago arabica, Medicago lupulina*, *Melilotus albus* and *Trifolium pratense*, had no difficulty to be recognized because of the density, length and shape of typical uniseriated eglandular trichomes. Neither did *Lotus tenuis* due to the large isodiametric epidermal cells with anticlinal cell wall pattern undulated, V-shaped with knobs thickening ornamentation (Dilcher, 1974). *Trifolium repens* presented some difficulties to be identified in the mixtures because of the density and distribution of the two types of trichomes in the leaflets. Glandular ones, scarce and scattered in the leaflets surface; and uniseriated hairs, more scarce and grouped at the base of the leaflets; so many fragments in ground material lacked hairs. Also, the shape of the epidermal cells was not enough to recognize this legume without mistake. In fact, all these species had isodiametric cells and straight anticlinal cell walls in the adaxial epidermis, and elongated to isodiametric cells with lightly undulated U-shaped anticlinal cell walls in the abaxial one. These were similar for *Medicago arabica* and *Medicago lupulina*, *Melilotus albus*, *Trifolium pratense* and *Trifolium repens*. This caused difficulties in the recognition of some mixtures with *Medicago arabica* and *Medicago lupulina*, and *Melilotus albus*, whereas we had no difficulties with *Lotus tenuis* and *Trifolium pratense*. In spite of the similar characteristics of the epidermal cells of the two last named species, the presence of the larger uniseriated trichomes in *Trifolium pratense* was very helpful in its recognition.

Discussion and Conclusions

The degree of undulation of the wall of the epidermal cells is characteristic of each species. But it can vary with environmental conditions (moisture, draught, shadow), and with phenologycal stage (Stace, 1965). The maximum growth of the undulations ceases before the cells (and leaf) reach total size (Stace, 1965). This could be the reason of the small differences in cell wall undulation between young and mature leaves.

Although all species presented papillae, this does not constitute a constant characteristic, as they were not present in all the leaves of the same species, so they cannot be used as a diagnostic character. The variation of the presence of papillae is related to environmental conditions (Fahn, 1990; Farooqui *et al*., 1997). Metcalfe & Chalk (1950) pointed out the abaxial epidermis papillose for *Lotus* and *Trifolium* species but they did not mention the presence of papillae in species of *Medicago* and *Melilotus* or in the adaxial epidermis of all these genera. Our results do not agree with Metcalfe & Chalk (1950) with respect to *Lotus tenuis* and *Trifolium repens* because we recorded the presence of papillae in the adaxial epidermis and they did in the abaxial one. But this coincides with what we found in *Trifolium pratense*. These differences are probably due to the fact that papillae are not a constant character.

Stomata position in relation to the rest of the epidermal cells was not useful to recognize fragments in ground material.

All six species presented typical uniseriated eglandular trichomes, with a variable number of short basal cells, accompanied by an elongated terminal cell. This type is typical in Papilionaceae according to Uphof (1962) and Metcalfe & Chalk (1950). But in this study it was necessary to establish with more details the characteristics offered by the cells which conform this trichomes in order to use them to recognize each of these species.

According to Arambarri & Colares (1993), *Lotus tenuis* showed epidermal cells with undulated anticlinal and convex external cell walls, and sunken stomata surrounded by three or four epidermal cells which form a triangular or trapezoidal space over them. Besides, we observed the undulated V-shaped with knobs thickening ornamentation, similar to Stenglein *et al*. (2003) description. These characteristics permit the recognition of *Lotus tenuis* without difficulties. Also, we observed papillae in some leaflets and the presence of typical uniseriated

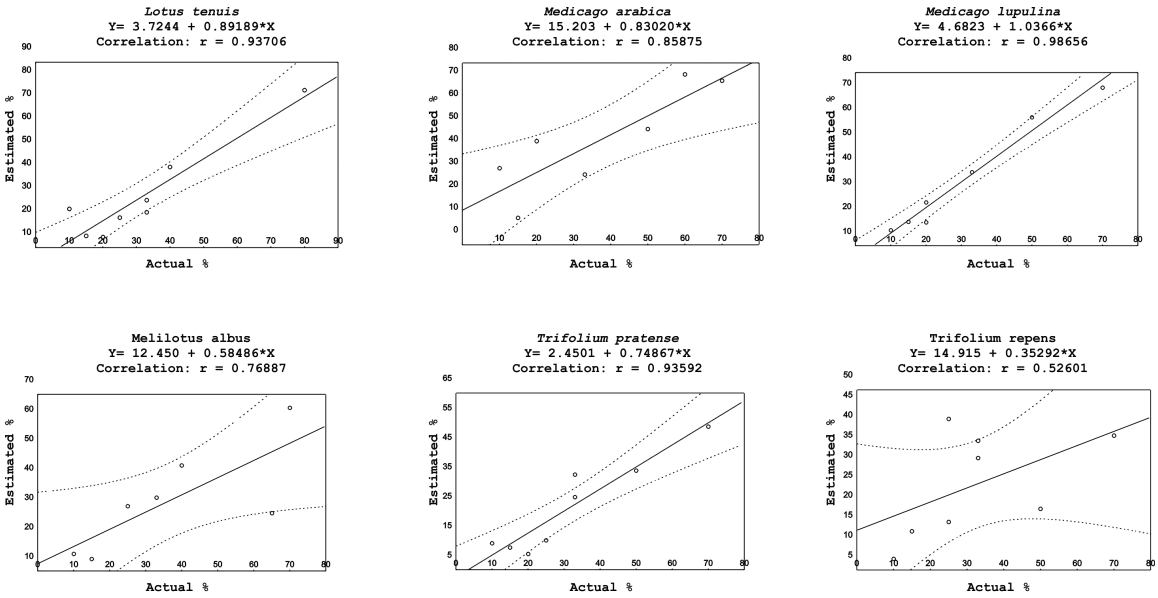

Fig. 3. Regression curves (95% confidence) of the studied species. Independent variable (X): percentage of the species in the mixture (actual %). Dependant variable (Y): quantified percentage of the species (estimated %).

Table 1. Leaflets epidermal cells qualitative microcharacters. adx=adaxial epidermis; abx=abaxial epidermis.

Species	Epidermal cell form		Anticlinal cell-wall patterns	
	adx	abx	adx	abx
Lotus tenuis	Isodiametric	Isodiametric	Lightly undulated, V-shaped with knobs	Undulated, V-shaped with knobs
Medicago arabica	Elongated to isodiametric	Isodiametric	Straight to undulated, U- shaped	Undulated, U-shaped
Medicago lupulina	Isodiametric	Isodiametric	Straight to lightly undulated	Lightly undulated
Melilotus indicus	Isodiametric	Isodiametric	Straight to lightly undulated	Undulated U- to V-shaped
Trifolium pratense	Isodiametric to elongated	Isodiametric to elongated	Straight to lightly undulated	Straight to undulated, U-to V-shaped with knobs
Trifolium repens	Isodiametric	Elongated to isodiametric	Straight	Lightly undulated U-shaped

Table 2. Minimum and maximum sizes of epidermal cells and stomata (μm) and stomatal density (number/mm^2). adx = adaxial epidermis; abx = abaxial epidermis (mean ± standard deviation).

Species	Epidermal cell				Stomata		Stomatal density	
	adx		abx					
	Length	Width	Length	Width	Length	Width	adx	abx
Lotus tenuis	27.5-75.0 (50.0±18.3)	30.0-92.5 (59.7±23.0)	65.0-120 (84.0±16.7)	50.0-70.0 (61.0±7.92)	22.5-27.5 (25.0±2.4)	20.0–22.5 (20.2±0.8)	153.0-223.0 (181.5±24.6)	127.0–172.0 (146.5±13.1)
Medicago arabica	15.0–57.5 (41.2±13.3)	12.5–42.5 (29.2±9.9)	25.0–62.5 (52.25±13.7)	20.0-50.0 (39.2±9.0)	20.0–22.5 (21,2±1.7)	12.5–17.5 (15.0±1.2)	286.6–407.6 (342.0±44.1)	248.4-407.6 (310.82±55.0)
Medicago lupulina	27.5–52.5 (40.0±8.57)	20.0–37.5 (30.50±5.50)	37.5-75.0 (49.7±12.3)	20.0–45.0 (33.75±6.9)	20.0–27.5 (23.0±3.5)	15.0–17.5 (15.2±7.9)	267.5-331.0 (293.6±22.6)	185.0-293.0 (252.23±32.1)
Melilotus albus	17.5–82.5 (48.0±19.14)	12.5–47.5 (34.7±10.4)	22.5–87.5 (57.5±18.8)	15.0–77.5 (42.5±18.7)	20.0–32.5 (26.2±4.6)	15.0–20.0 (17.5±1.7)	152.8–197.5 (168.1±14.5)	76.4–146.5 (111.5±27.7)
Trifolium pratense	30.0-55.0 (44.2±7.9)	22.5–42.5 (36.50±8.09)	30.0-80.0 (55.7±12.7)	22.5–57.5 (43.5±10.5)	15.0–22.5 (19.1±2.5)	12.5-20.0 (13.5±2.4)	210.0-293.0 (249.0±23.3)	185.0-369.0 (252.86±54.2)
Trifolium repens	27.5–50.0 (38.0±7.62)	17.5–37.5 (28.0±5.9)	40.0-75.0 (61,7±10.4)	30.0–50.0 (37,7±6.9)	20.0–30.0 (25.2±3.8)	12.5–20.0 (17.2±3.0)	223.0–344.0 (288.5±46.8)	102.0–185.0 (148.4±28.3)

Table 3. Typical eglandular uniseriated trichomes: Cells sizes (μm), density (number/mm^2) and number of basal cells. adx = adaxial epidermis; abx = abaxial epidermis, (mean ± standard deviation).

Species	Distal cell		Medial cell		Proximal cell		Density		N of basal cells
	Lengt	Width	Lengt	Width	Lengt	Width	abx	adx	
Lotus tenuis	175.0- 400.0 (298.5±79.8)	15.0–20.0 (17.7±1.8)	7.5–17.5 (12.0±3.7)	15.0-20.0 (17.0±1.6)	12.5-25.0 (15.2±3.8)	12.50-25.0 (18.0±4.2)	Grouped on petiole & leaflet base		5- 6 (5.3±0.8)
Medicago arabica	325.0– 550.0 (442.7±73.5)	10.0–17.5 (14.2±2.4)	12.5-22.5 (18.2±3.5)	10.0-17.5 (13.5±3.8)	10.0-22.5 (16.0±5.0)	15.0-22.5 (18.7±3.2)	6.3-12.7 (8.3±3.0)	Absent	7-10 (8.2±1.3)
Medicago lupulina	252.0- 675.0 (411.2±124.6)	10.0–15.0 (12.5±1.2	10.0–12.5 (11.7±1.3)	12.5-17.5 (15±1.6)	12.5-20.0 (14.5±5.9)	22.5-30.0 (25.7±2.6)	19.0-44.5 (33.1±8.9)	6.36-25.4 (15.9±5.4)	6- 8 (7.8±0.8)
Melilotus albus	175.0– 375.0 (267.5±67.8)	10.0–12.5 (11.7±1.2)	10.0–12.5 (12.0±1.0)	10.0-12.5 (10.2±0.8)	7.5-22.5 (13.2±5.9)	7.5-15.0 (11.5±2.9)	6.3-25.4 (11.5±7.2)	Absent	6-8 (6.2±1.0)
Trifolium pratense	612.5-1325.0 (1108.7±193.5)	20.0–37.5 (26.0±4.9)	Absent	Absent	22.5-32.5 (27.5±3.5)	45.0-62.5 (53.7±6.8)	6.3-12.7 (8.1±2.6)	0.00-6.36 (3.2±3.3)	10-16 (12.9±1.8)
Trifolium repens	325.0- 500.0 (439.0±68.5)	12.5–20.0 (16.5±1.6)	12.5–15.0 (13.2±1.0) sometimes absent	12.5-20.0 (16.2±1.3) sometimes absent	22.5-25.0 (26.4±2.2)	8.7-17.5 (12.3±2.2)	Grouped on petiole & leaflet base		5-6 (5.5±0.4)

Table 4. Glandular trichomes: Minimum and maximum sizes of cells (μm), density (number/mm^2) and number of head and basal cells. adx=adaxial epidermis; abx = abaxial epidermis, (mean ± standard deviation).

Species	Head		Foot				Prox. cell		N of basal cells	Density		N of head cells
			Cell 1		Cell 2							
	Length	Width	Length	Width	Length	Width	Length	Width		abax.	adax.	
Lotus tenuis	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Medicago Arabica	45.0–75.0 (58.7±8.7)	20.0–25.0 (23.0±2.0)	20.0–37.5 (26.4±5.5)	10.0–12.5 (11.5±1.15)	absent	absent	10.0–17.5 (15.0±2.6)	15.0-17.5 (17.5±1.7)	5.0-10.0 (6.7±1.6)	6.36-12.7 (5.7±4.7)	absent	5-7
Medicago lupulina	30.0–45.0 (35.5±6.0)	15.0–20.0 (17.5±1.7)	20.0–40.0 (26.5±5.3)	7.5 (7.5±0.0)	absent	absent	7.5-12.5 (11.5±2.7)	7.5-17.5 (11.5±2.7)	6.0- 8.0 (7.6±0.8)	0.00-19.0 (4.5±6.0)	0.0-12.7 (1.3±4.0)	3-4
Melilotus albus	27.5–40.0 (34.2±3.5)	17.5–27.5 (24.5±3.3)	10.0–17.5 (14.7±2.7)	10.0–15.0 (12.0±2.0)	10.0–17.5 (10.2±5.9)	10.0–15.0 (10.0±5.4)	7.5-15.0 (11.7±2.6)	10.0-27.5 (14.0±5.3)	4.0- 9.0 (6.3±1.5)	0.00-12.7 (3.18±4.5)	absent	1-4
Trifolium pratense	37.5–57.5 (48.0±6.4)	25.0–37.5 (27.9±3.4)	15.0–37.5 (30.2±7.5)	17.5–22.5 (18.5±1.7)	absent	absent	20.0 (20.0±0.0)	17.5 (17.5±0.0)	6.0 (6.0±0.0)	On margin but not ever		4
Trifolium repens	40.0–70.0 (58.0±8.3)	17.5–25.0 (22.7±2.9)	15.0–20.0 (18.5±2.9)	12.5–17.5 (13.5±1.7)	absent	absent	7.5-12.5 (11.0±1.7)	10.0-20.0 (15.2±3.2)	5.0- 8.0 (6.1±0.7)	6.3±0.0	absent	4-6

eglandular trichomes in the petiolules. The presence of papillae was not mentioned by Arambarri & Colares (1993) nor by Stenglein *et al*. (2003), but the presence of typical uniseriated egalndular trichomes coincides with Stenglein *et al*. (2003).

Although *Trifolium repens* has epidermal characteristics that permit its recognition, over-estimation or under-estimation in its quantification in the hand-composed mixtures depended on the other species present. By another hand, it must be considered that it is very improbable that the six species appear together at the same place as available for herbivores.

We can conclude that the main characteristics that help us in the recognition of these species are the form of the anticlinal epidermal cell walls, and the density, dimensions and form of the typical uniseriated eglandular trichomes. According to the epidermis descriptions and the statistical analysis performed, it is possible to recognize these species when present in herbivore faeces or in digestive tract contents. Furthermore, these descriptions were valuable to identify legumes in a rhea's fecal diet study (Comparatore & Yagueddú, 2007).

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