Comparative root anatomy of *Duchesnea indica*, *Fragaria vesca* and *Potentilla tucumanensis* (Rosaceae) in Tucumán province, Argentina

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Albornoz P., Arias M., Castagnaro A. & Díaz Ricci J. C. 2007. — Comparative root anatomy of *Duchesnea indica*, *Fragaria vesca* and *Potentilla tucumanensis* (Rosaceae) in Tucumán province, Argentina. *Adansonia*, sér. 3, 29 (2): 255-267.

ABSTRACT
We report the morphological and anatomical characterization of the primary and secondary structures of root systems in *Duchesnea indica* (Andrews) Focke, *Fragaria vesca* L. and *Potentilla tucumanensis* Castagnaro & Arias, three species that show a main tap-root with a variable branching pattern. Mother plants of *F. vesca* present main roots with a primary structure of the diarch type, a secondary structure with a polyderm, and adventitious roots from diarch to pentarch stele. Mother plants of *D. indica* and *P. tucumanensis* do not form adventitious roots, the primary structure is a diarch, and the secondary structure lacks a polyderm. Daughter plants of *D. indica* and *F. vesca* form adventitious roots with variable steles, from diarch to polyarch. Tracheids were only observed in *F. vesca* and *P. tucumanensis*. Our results show that the number of protoxylem poles, and the occurrence of tracheids and polyderm constitute valuable diagnostic characters for the identification of these species.

KEY WORDS
Duchesnea, *Fragaria*, *Potentilla*, root, anatomy, morphology, polyderm, tracheids, Argentina.

RÉSUMÉ
Anatomie comparée des racines de Duchesnea indica*,* Fragaria vesca *et* Potentilla tucumanensis *(Rosaceae) dans la province de Tucumán, Argentine.*
Ce travail caractérise la morphologie et l'anatomie des structures primaire et secondaire du système racinaire de *Duchesnea indica* (Andrews) Focke, *Fragaria vesca* L. et *Potentilla tucumanensis* Castagnaro & Arias. Morphologiquement, les trois espèces montrent une racine principale pivotante avec un mode de ramification variable. Les plantes mères de *F. vesca* présentent des racines séminales à structure primaire diarche et secondaire avec polyderme, et des racines adventives diarches à pentarches. Les plantes mères de *D. indica* et *P. tucumanensis* ne forment pas de racines adventives, la structure primaire est diarche et la secondaire sans polyderme. Les plantes filles de *D. indica* et *F. vesca* forment des racines adventives diarches à polyarches. Nous n'avons observé de trachées que chez *F. vesca* et *P. tucumanensis*. Nos résultats montrent que le nombre de pôles de protoxylème et la présence de trachées et de polyderme constituent des éléments diagnostiques pour l'identification de ces espèces.

MOTS CLÉS
Duchesnea, *Fragaria*, *Potentilla*, racine, anatomie, morphologie, polyderme, trachées, Argentine.

INTRODUCTION

The family Rosaceae Jussieu (1789), typical of the northern hemisphere, comprises 120 genera and 3370 species of cosmopolitan distribution, and is divided into four subfamilies with approximately 15 tribes (Zardini 1973). Three genera, *Duchesnea* Sm., *Fragaria* L. and *Potentilla* L., are included in the subtribe Potentillinae. According to Zardini (1999) there are 19 genera and 74 species (including 11 endemic species) of Rosaceae in Argentina, with *Duchesnea indica* (Andrews) Focke occurring in the Delta del Paraná and the northwestern forest; and *Fragaria vesca* L. in Ushuaia and Tucumán. Steibel & Troiani (1999) reported *F. vesca* as a rare species in La Pampa province, and Castagnaro *et al.* (1998) indicated the presence of *Potentilla tucumanensis* Castagnaro & Arias in northwestern Argentina.

In Tucumán these wild strawberry-like species constitute a natural genetic resource. *Duchesnea indica* and *Fragaria vesca* are perennial, stoloniferous, trifoliate creeping herbs that grow in forest understory and in open habitats and pastures (Arias *et al.* 1996, 2004). *Potentilla tucumanensis* is an erect, trifoliate, annual herb that grows in disturbed environments (Arias *et al.* 1998). Similarities among these species were evaluated in our laboratory by using morphological, anatomical and molecular characters (Ontivero *et al.* 2000).

Although the literature about the morphology and anatomy of over ground parts of these species is abundant, reports of their root features are scarce and most concern the anatomy of different varieties of cultivated strawberries (White 1927, 1929; Mann 1930; Nelson & Wilhem 1957). Metcalfe & Chalk (1950) published some information on the root anatomy of Rosaceae and referred to the studies carried out by White (1927), who analyzed the relation between adventitious and fibrous roots in different varieties of strawberry.

Darrow (1966) mentioned the variability and plasticity observed in the root system of *Fragaria ananassa* in relation to the environment. Later, Naruhashi & Ishizu (1992) conducted a comparative anatomical study among *Duchesnea chrysantha*, *D. indica* and their hybrids; showing that in *D. indica* the roots grow from rhizome and that adventitious roots sprouts from each node of a runner.

Due to the paucity of information found in the available literature on root anatomy, the aim of this work is to analyze the root morphology and anatomy of *Duchesnea indica*, *Fragaria vesca* and *Potentilla tucumanensis*. The seasonal and environmental variation of their morphology in Tucumán, Argentina is also studied.

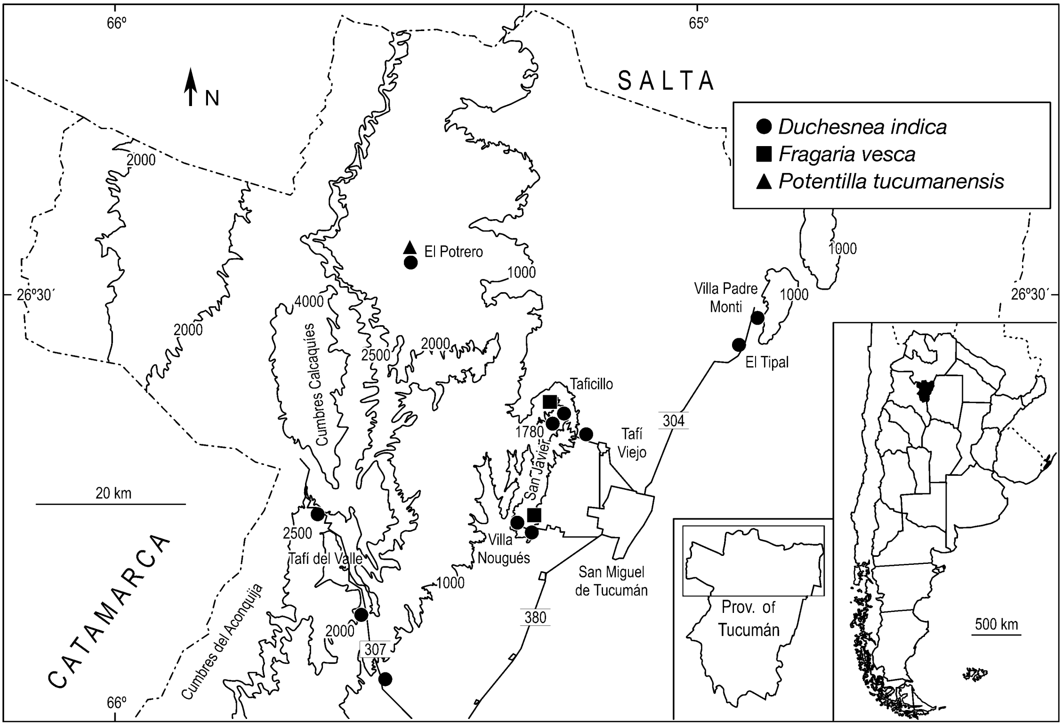

FIG. 1. — Locations of sampling collection places of *Duchesnea indica* (Andrews) Focke, *Fragaria vesca* L. and *Potentilla tucumanensis* Castagnaro & Arias in Tucumán, Argentina.

MATERIALS AND METHODS

Root samples were collected in different seasons, during the period 1999-2003, in the months of February, May, August and November. Observations were made at different altitudes in forest, woodland, pasture and disturbed environments in the following localities of the province of Tucumán: Tafí del Valle, El Potrero, Tafí Viejo, Villa Nougués, Villa Padre Monti and El Tipal (Fig. 1). Seedlings were obtained from achenes germinated under axenic conditions whose surface was previously sterilized with hypochloride (1/1 v/v with water, 1 min) and washed three times with sterile distilled water. In the case of *Fragaria vesca* and *Duchesnea indica*, achenes were further treated with concentrated sulphuric acid (95%) for 7 min and washed 10 times with sterile distilled water. Sterile and scarified achenes were put in sterile Petri dishes containing filter paper soaked with sterile distilled water and incubated in a wet chamber at 70% RH, 28 °C and 14 hours light cycle. After two or three weeks germinated achenes were moved into pots containing natural (not sterile) soil coming from the sample location.

From each locality and altitude we randomly selected five individuals from which roots were immediately fixed in FAA (1/1/8 v/v/v, formaline:glacial acetic acid:ethanol 80%). Specimens prepared from these individuals were deposited in the Miguel Lillo Herbarium (LIL). Cross and longitudinal sections 15 µm in thickness were made freehand and with a Leitz MINOT microtome (Johansen 1940). Sections were stained with safranin, and double stained with safranin-fast green and cresyl violet (D'Ambrogio de Argüeso 1986; Dizeo de Strittmatter 1980). In the case of fresh material, samples were mounted in glycerin:water (1/1 v/v) and Canada Balsam was used to make permanent preparations. All prepared material is preserved in the histological library of the Institute of Plant Morphology at Fundación

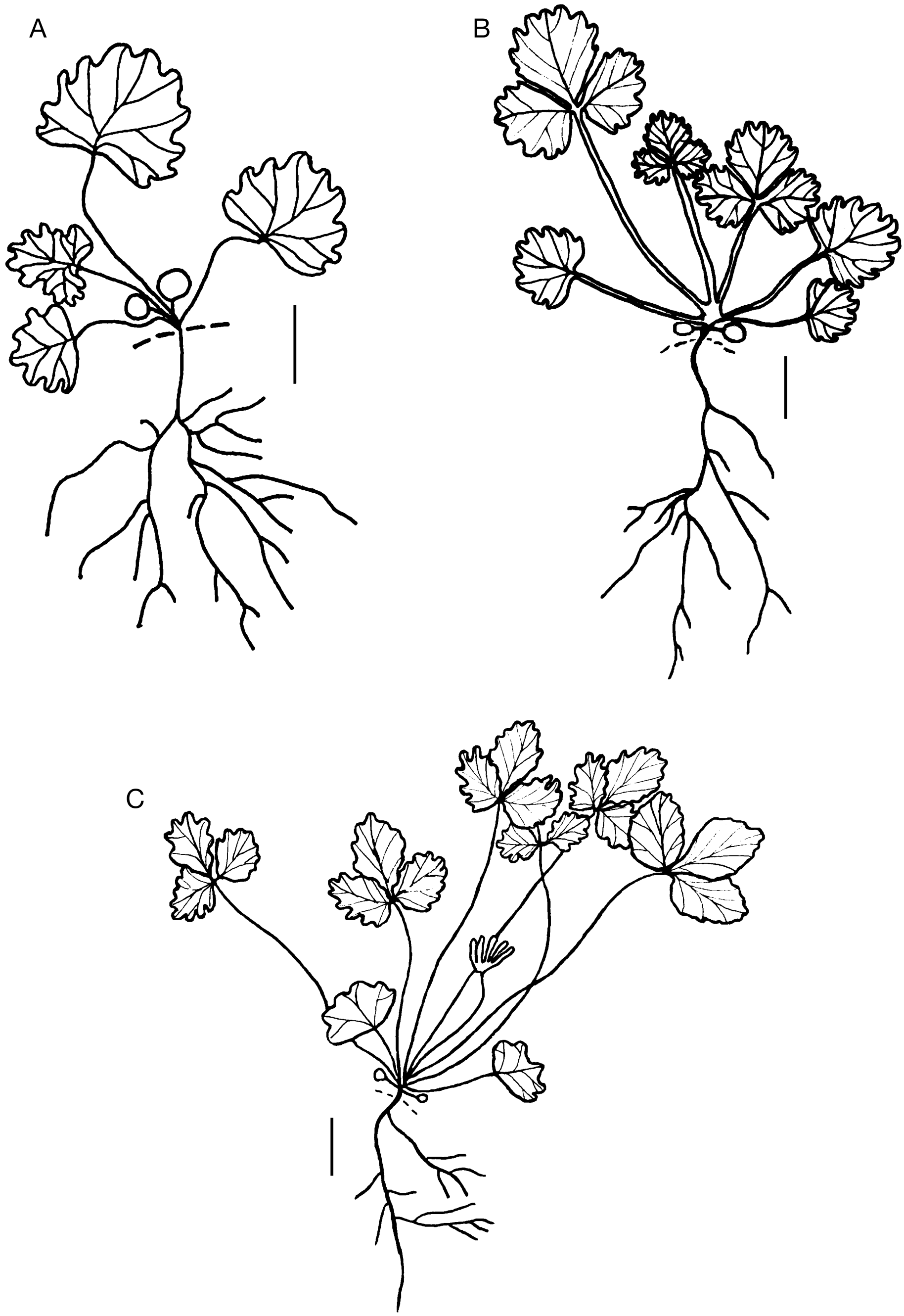

FIG. 2. — Plants grown in laboratory: **A**, *Duchesnea indica* (Andrews) Focke; **B**, *Fragaria vesca* L.; **C**, *Potentilla tucumanensis* Castagnaro & Arias. Scale bars: A, 0.5 cm; B, C, 1 cm.

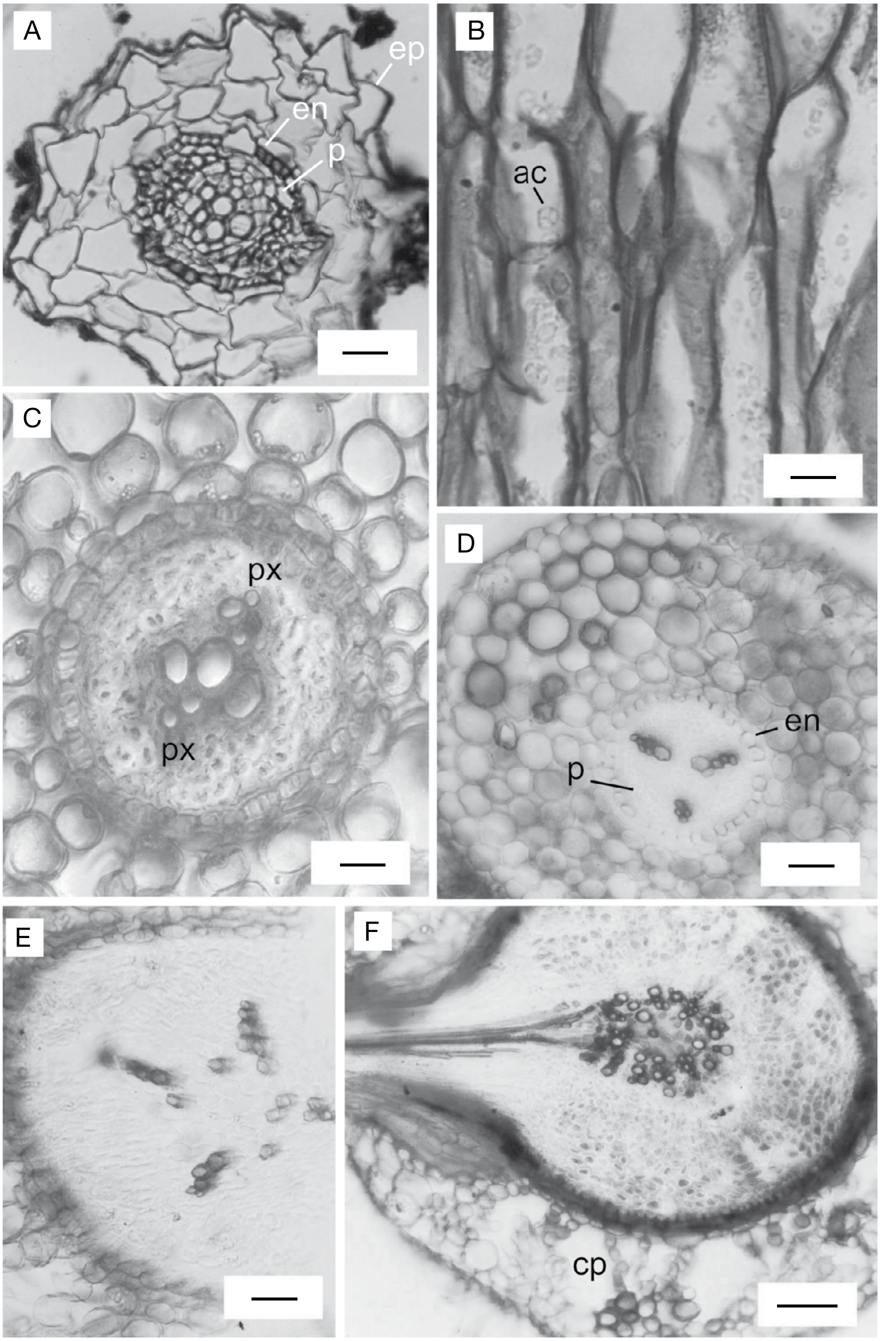

FIG. 3. — *Duchesnea indica* (Andrews) Focke: **A**, transversal section of root with primary structure; **B**, longitudinal section of the cortical parenchyma with complex starch; **C**, diarch stele; **D**, triarch stele; **E**, tetrarch stele; **F**, early secondary structure. Abbreviations: **ac**, complex starch; **cp**, cortex of the primary structure; **en**, endodermis; **ep**, epidermis; **p**, pericycle; **px**, protoxylem. Scale bars: A, C-E, 20 µm; B, 10 µm; F, 100 µm.

TABLE 1. — Field and laboratory anatomical characters of *Duchesnea indica* (Andrews) Focke, *Fragaria vesca* L. and *Potentilla tucumanensis* Castagnaro & Arias.

		D. indica		*F. vesca*		*P. tucumanensis*	
		1st structure	2nd structure	1st structure	2nd structure	1st structure	2nd structure
Field	Mother plants	diarch	without polyderm	di- tri-, tetr-, pentarch	with polyderm	diarch	without polyderm
	Daughter plants	di-, tri-, tetrarch	without polyderm	di- tri-, tetr-, pentarch	with polyderm	absent	
Laboratory	Mother plants	diarch	without polyderm	di- tri-, tetr-, pentarch	with polyderm	diarch	without polyderm
	Daughter plants	diarch	without polyderm	di-, tri-, tetr-, pent-, polyarch	with polyderm	absent	

Miguel Lillo. Microphotographs were taken with a MC 80 camera mounted on a Zeiss Axiolab light microscope.

RESULTS

Morphological and anatomical studies were made from the samples based on material collected in field and compared with samples grown in the greenhouse.

MORPHOLOGICAL ANALYSES

Duchesnea indica, *Fragaria vesca* and *Potentilla tucumanensis* present a main root from which variable lateral branching emerges, depending on the environment in which the plant was found. *Fragaria vesca* shows adventitious roots formed above the main root of the mother plant, which are absent in *D. indica* and *P. tucumanensis*. *Duchesnea indica* and *F. vesca* produce runners that generate daughter plants with adventitious roots. The foliar sequence for *D. indica* and *F. vesca* is epigeous photosynthesizing cotyledons, three unifoliate eophyls, and spiral packed metaphyls (Fig. 2A, B). A similar sequence is seen in *P. tucumanensis* except that it contains only two eophyls (Fig. 2C).

ANATOMICAL ANALYSES OF *D. INDICA*

In cross section the roots of this species present an unistratified epidermis, the cortical parenchyma is formed by isodiametric cells, the endodermis shows Caspary strips, and the pericycle is unistratified (Fig. 3A). During the unfavorable season (e.g., month of May), the parenchymatic tissue produces complex grains of starch as storage material (Fig. 3B). The main root, its branching and the adventitious roots present a primary structure of the diarch type (Fig. 3C). In the field we also found triarch and tetrarch primary structures from the third daughter (Table 1; Fig. 3D, E) and in both greenhouse and field plants, we observed that the primary cortex remains tightly bound to the juvenile peridermis (Fig. 3F).

The secondary plant body is typical, and is similar in individuals coming from the greenhouse and the field. Cross sections show a peridermis (suber, phellogen and phelloderm), cortical parenchyma and vascular system (phloem, cambium, xylem) (Fig. 4A).

In longitudinal sections we observed short trachea segments with an appendix, simple oblique to horizontal plate, scalariform and reticulated thickening, and opposing punctuation (Fig. 4B, C).

ANATOMICAL ANALYSES OF *F. VESCA*

In cross section the epidermis and the exodermis are unistratified (Fig. 5A, B), the cortical parenchyma is formed by isodiametric cells (Fig. 5B), the endodermis may have very thick walls (Fig. 5C), and the 1-2-layered pericycle may become lignified (Fig. 5D, F). An amyliferous sheath located outside the endodermis may be present. During the unfavorable season, the parenchymatic tissue

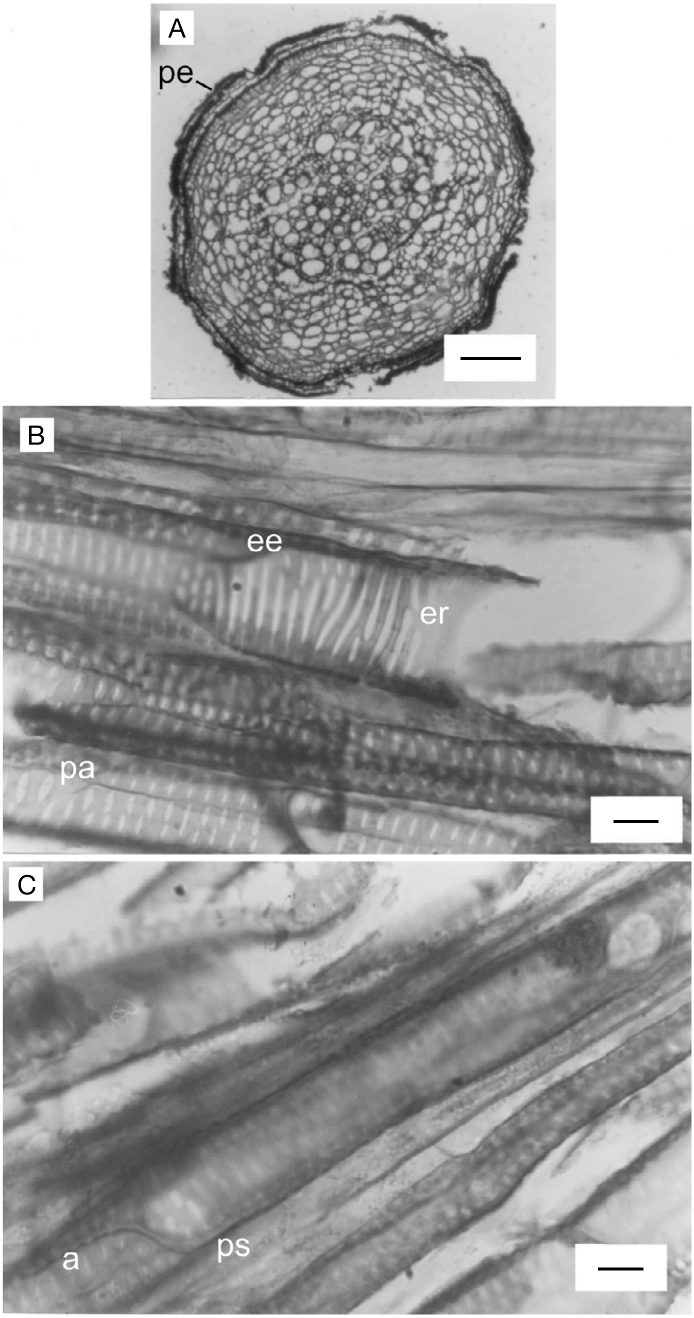

FIG. 4. — *Duchesnea indica* (Andrews) Focke: **A**, transversal section of root with secondary structure; **B**, longitudinal section, trachea scalariform segment and areolate punctuation; **C**, trachea segment with oblique simple plate and appendix. Abbreviations: **a**, appendix; **ee**, scalariform thickened; **er**, reticulate thickened; **pa**, areolate punctuation; **pe**, periderm; **ps**, simple plate. Scale bars: A, 100 µm; B, C, 10 µm.

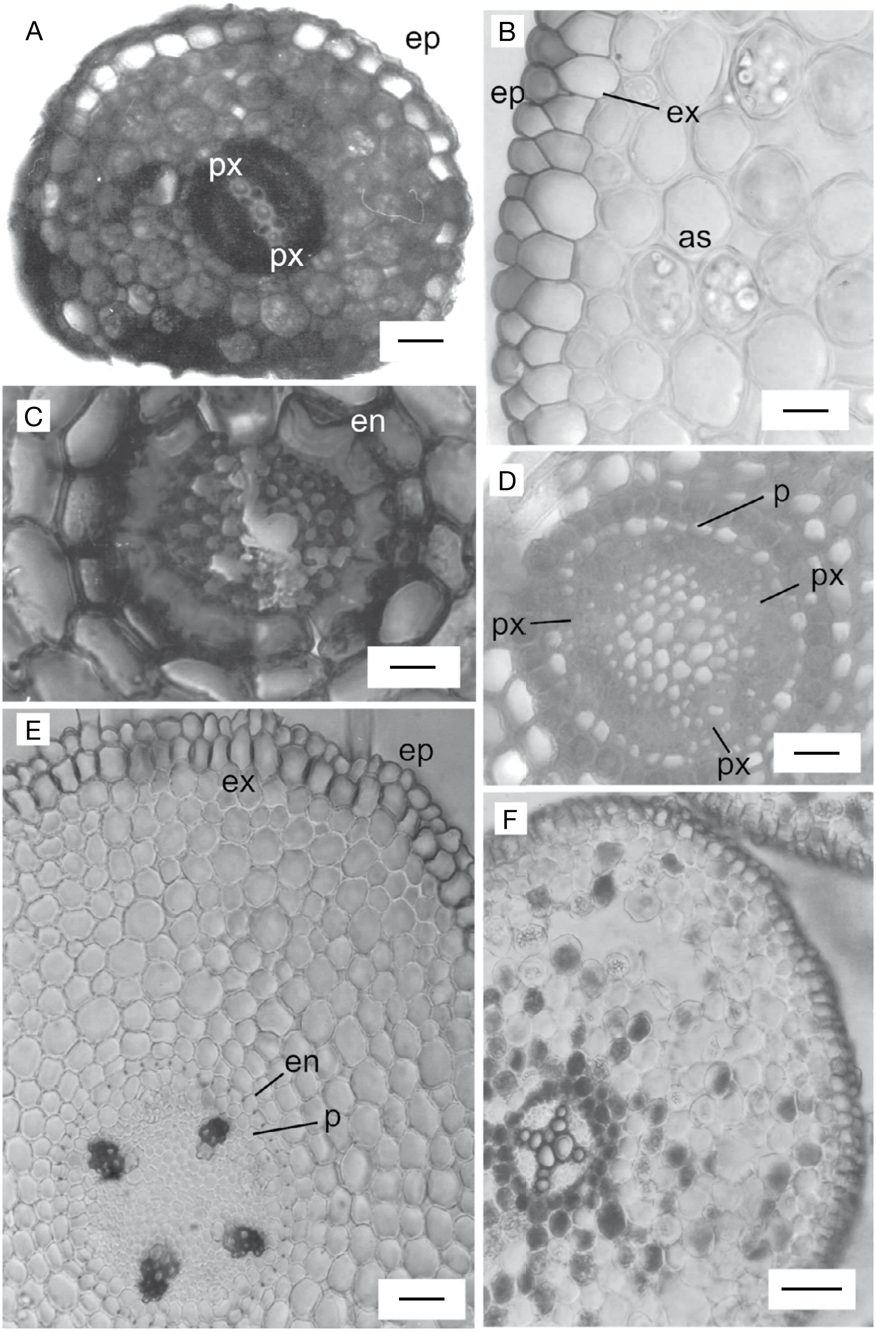

FIG. 5. — *Fragaria vesca* L.: **A**, transversal section of root, primary structure diarch; **B**, simple starch in cortical parechyma; **C**, endodermis with thick walls; **D**, triarch stele with lignified pericycle; **E**, tetrarch stele with not lignified pericycle; **F**, tetrarch stele in advanced state of development. Abbreviations: **as**, simple starch; **en**, endodermis; **ep**, epidermis; **ex**, exodermis; **p**, pericycle; **px**, protoxylem. Scale bars: A, B, E, 20 µm; C, D, 10 µm; F, 100 µm.

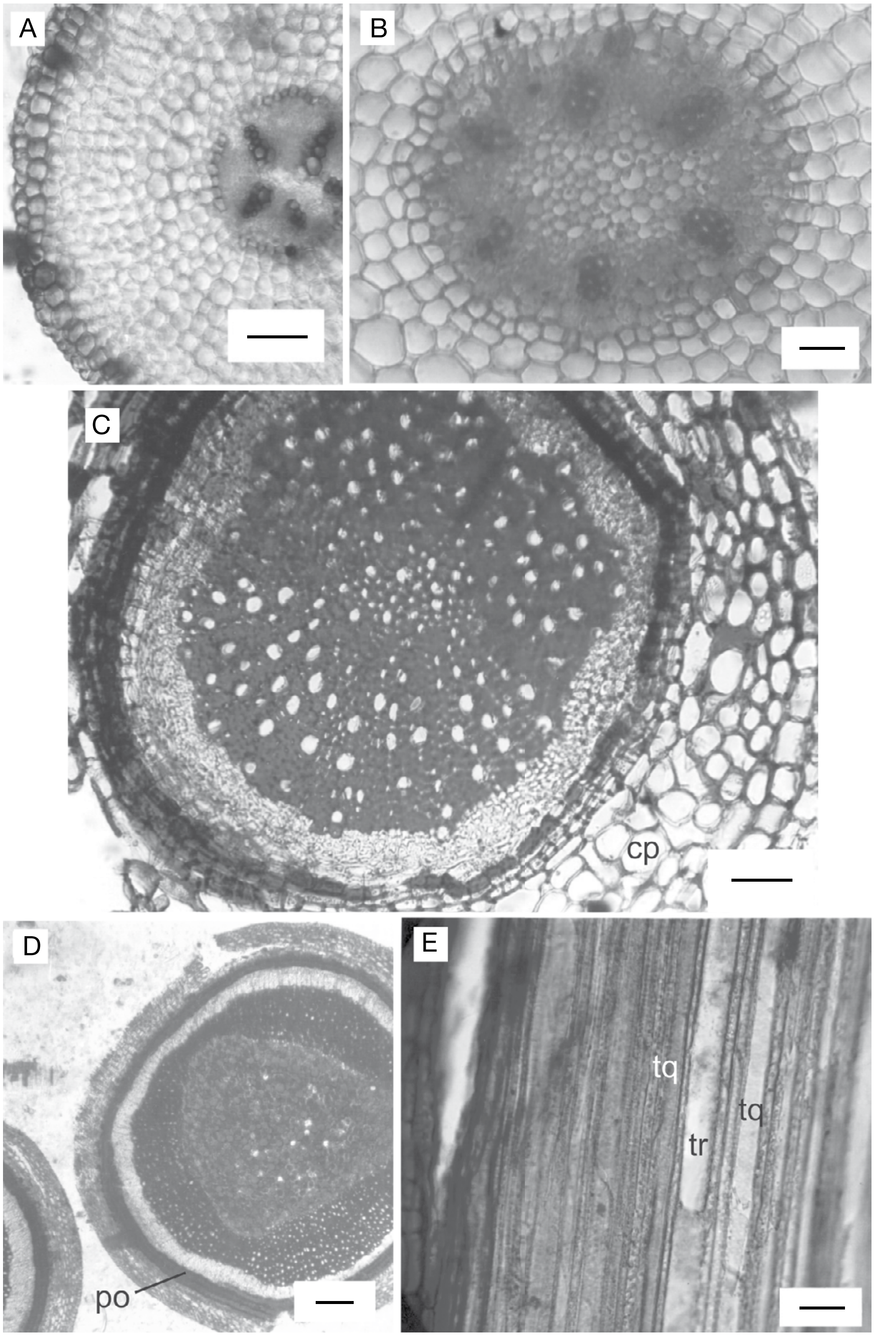

FIG. 6. — *Fragaria vesca* L.: **A**, transversal section of root, pentarch stele; **B**, poliarch stele; **C**, early secondary structure; **D**, secondary structure with polyderm; **E**, longitudinal section, trachea segment with areolate punctuation and tracheids. Abbreviations: **cp**, cortex of primary structure; **po**, polyderm; **tq**, tracheid; **tr**, trachea. Scale bars: A, C, 70 µm; B, E, 40 µm; D, 20 µm.

produces simple grains of starch as storage material (Fig. 5B).

The primary structure of the main root, its branches and adventitious roots is of the diarch, triarch, tetrarch and pentarch type (Figs 5A, D, F; 6A). Additionally, we found a primary structure of the polyarch type in plants grown in the greenhouse (Table 1; Fig. 6B). We frequently observed, in plants grown both in the field and the greenhouse, secondary structures in which the primary cortex remains attached to the polydermis while the latter is still in formation (Fig. 6C).

The secondary structure is similar in plants coming from the field and the greenhouse, presenting a characteristic polyderm, cortical parenchyma, and vascular system (phloem, cambium, xylem) (Fig. 6D).

In segments of longitudinal sections, we observed trachea with appendices, oblique to horizontal simple plates, and alternate punctuation. We also detected the presence of numerous tracheids with areolate punctuations in a line (Fig. 6E).

Anatomical analyses of *P. tucumanensis*

Cross sections of roots present a 1-layered epidermis, cortical parenchyma with isodiametric cells, endodermis with Caspary strips, and an unistratified pericycle (Fig. 7A). The main root and its branches in plants coming from the field and the greenhouse have a primary structure of the diarch type. Early secondary structure with the remains of primary cortex still attached to the endodermis was also observed (Fig. 7B).

Greenhouse and field plants showed a characteristic secondary structure with peridermis (suber, phellogen and phelloderm), cortical parenchyma, and vascular system (phloem, cambium, xylem) (Fig. 7C).

In longitudinal section we observed trachea segments with oblique to horizontal simple plates, scalariform and reticulated thickening, and opposite or alternate areolate punctuation. We detected the presence of numerous tracheids (Fig. 7D).

In all three of the species studied endomycorrhiza were observed in the roots.

Ecological considerations

Duchesnea indica, *F. vesca* and *P. tucumanensis* present a diarch primary structure regardless of the environment or the month of collection. However, *D. indica* presented additionally triarch and tetrarch structures during the months of February and May in forest, woodland and pasture environments (1480-1860 m) at Tafí del Valle, Taficillo, Villa Nougués, Potrero and El Tipal (840 m). In February, *F. vesca* had a triarch structure in pasture environments (1780 m) at Taficillo. Nevertheless, during the months of February and May, in Villa Nougués (1230 m), tri-, tetra- and pentarch primary structures were observed.

During the month of November in different environments (e.g., forest, woodland, pasture and disturbed environments) we observed a typical secondary structure in the three species studied.

In the case of individuals of *D. indica* and *F. vesca* growing near streams, aerenchyma in the cortex of the primary structure was also found. This may be directly related to the specific environment in the latter species.

DISCUSSION

Our results show that the most variable species with regard to the growth pattern of its primary structure is *F. vesca*, in contrast to what was observed in *P. tucumanensis* and *D. indica*.

The polyderm is present in the secondary structure in wild and cultivated species (e.g., all cultivars of *Fragaria* × *ananassa* studied), suggesting that it may constitute a diagnostic character of the genus *Fragaria* as shown in Table 1.

Duchesnea indica presents lateral roots that sprout from the main root of the mother plant and from the rhizome as reported by Naruhashi & Ishizu (1992). In the anatomical root characterization made by the same authors, they described an early secondary structure but did not mention the type of stele of the primary structure. *Fragaria vesca*, on the other hand, presents lateral and adventitious roots emerging from the principal root of the mother plant in a manner similar to that observed in other wild species and cultivated strawberry varieties (White 1927; Mann 1930; Nelson & Wilhem 1957).

The presence of tracheids in the vascular system of *F. vesca* and *P. tucumanensis* can be interpreted as an adaptive response to dry environments.

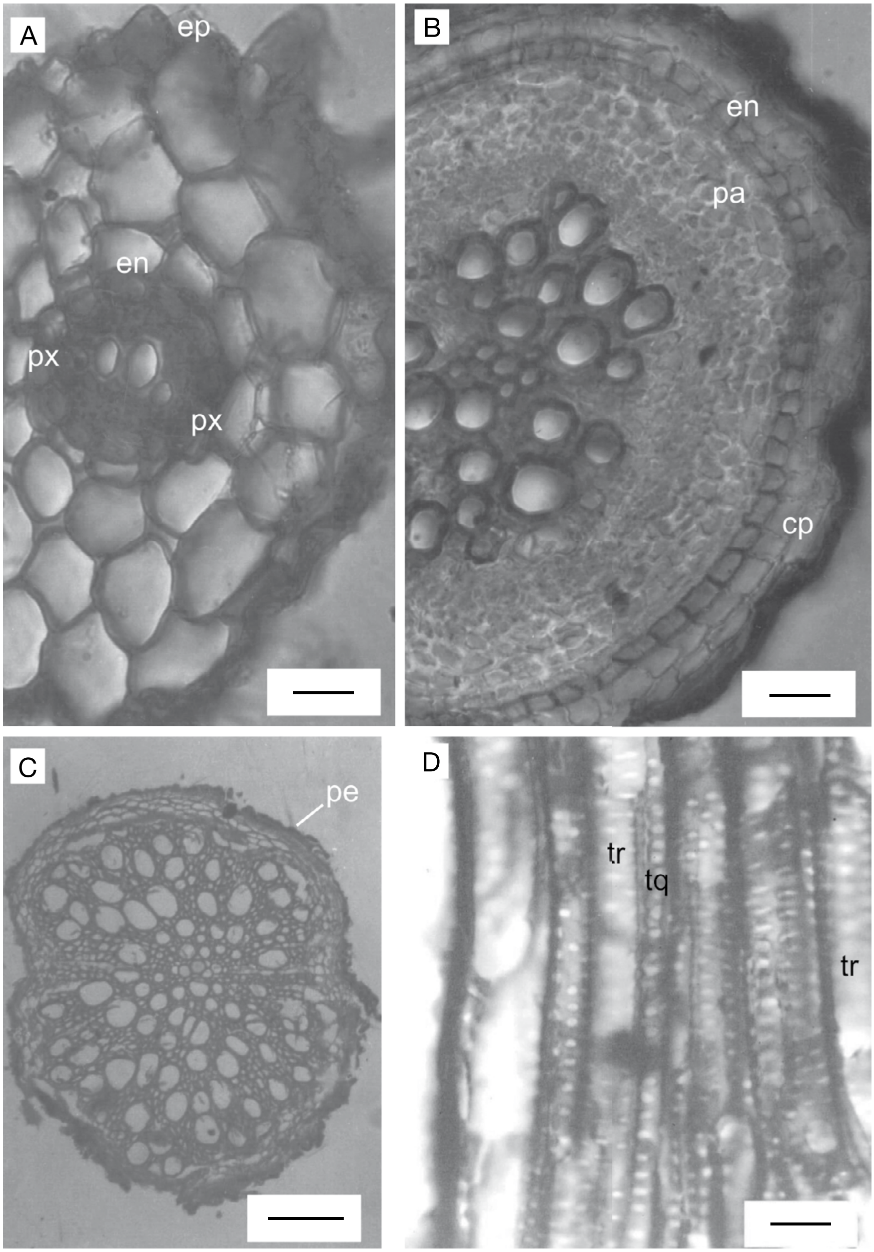

FIG. 7. — *Potentilla tucumanensis* Castagnaro & Arias: **A**, transversal section of root, diarch primary structure; **B**, early secondary structure; **C**, secondary structure; **D**, longitudinal section, trachea segment with areolate punctuation and tracheids. Abbreviations: **en**, endodermis; **ep**, epidermis; **cp**, cortex of the primary structure; **pa**, parenchyma; **pe**, peridermis; **px**, protoxylem; **tq**, tracheid; **tr**, trachea. Scale bars: A, D, 10 µm; B, 20 µm; C, 100 µm.

The tri-, tetra- and pentarch primary structure, common in adventitious roots during the months of February and May in forest, woodland, pasture and disturbed environments, may be the result of an active stolonization stage in plants during previous months.

White (1929) and Darrow (1966), have observed the restricted distribution area of *F. chiloensis*, suggesting that presence of mycorrhizal fungi may contribute to the dissemination of this species. In this paper we report the presence of symbiotic associations of vesicular-arbuscular mycorrhizal (VAM) fungi associated with the three species examined. Further studies are currently in progress in our laboratory to characterize these fungi more thoroughly.

Acknowledgments
This paper was partially supported by grants of CIUNT 26/D346, PICTO 759 and Fundación Miguel Lillo. AC and JCDR are members of CONICET. We wish to thank Mrs Jaume and Mr Sánchez for the preparation of the figures; Dr Gallardo and Lic Podaza for greenhouse guidance, and Mr Carabajal and Dr Tracanna de Albornoz for translation of the abstract.

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Submitted on 23 March 2006;
accepted on 7 September 2007.

APPENDIX

Examined material; the specimens studied are deposited at LIL Herbarium.

Duchesnea indica **(Andrews) Focke**
Argentina. Prov. Tucumán, Monteros, Tafí del Valle, Pastizal km 63, 2300 m, 26°49'S, 65°42'W, 13.X.2003, fl., *Albornoz et al. 22.* — *Ibid.*, 13.X.2003, fl., *Albornoz et al. 23.* — Monteros, Camino Tafí del Valle, Apeadero Muñoz, Selva, 1610 m, 26°59'S, 65°39'W, 8.VIII.1999, fl., *Albornoz 1.* — *Ibid.*, 13.X.2003, fr., *Albornoz et al. 16.* — *Ibid.*, 13.X. 2003, fl., *Albornoz et al. 17.* — *Ibid.*, 13.X.2003, fl., *Albornoz et al. 18.* — *Ibid.*, 13.X.2003, fl., *Albornoz et al. 19.* — *Ibid.*, 13.X.2003, fl., *Albornoz et al. 20.* — *Ibid.*, 13.X.2003, fl., *Albornoz et al. 21.* — Monteros, Selva km 23, 700 m, 26°04'S, 65°39'W, 13.X.2003, fr., *Albornoz et al. 12.* — *Ibid.*, 13.X.2003, fr., *Albornoz et al. 13.* — *Ibid.*, 13.X.2003, fr., *Albornoz et al. 14.* — *Ibid.*, 13.X.2003, fl., fr., *Albornoz et al. 15.* — *Ibid.*, 13.X.2003, fl., fr., *Albornoz et al. 24.* — Villa Padre Monti, al lado del río, 935 m, 26°30'S, 64°59'W, 12.VIII.1999, fl., *Albornoz 2.* — Trancas, Potrero, al lado del río, 1500 m, 26°26'S, 65°33'W, 31.VIII.1999, fl., *Albornoz 5.* — *Ibid.*, 1.X.2003, fl., *Albornoz et al. 7.* — *Ibid.*, 1.X.2003, fl., *Albornoz et al. 8.* — *Ibid.*, 1.X.2003, fl., fr., *Albornoz et al. 9.* — Tafí Viejo, Taficillo, Pastizal, 1770 m, 26°41'S, 65°20'W, 29.X.2003, fl., fr., *Albornoz et al. 27.* — *Ibid.*, 29.X.2003, fl., fr., *Albornoz et al. 30.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 31.* — Tafí Viejo, Taficillo, Pastizal 1750 m, 26°41'S, 65°20'W, 29.X.2003, fl., fr., *Albornoz et al. 28.* — *Ibid.*, 29.X.2003, fl., fr., *Albornoz et al. 29.* — Tafí Viejo, Taficillo (Bosque 1600 m), 26°41'S, 65°19'W, 29.X.2003, fl., fr., *Albornoz et al. 32.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 33.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 34.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 35.* — Tafí Viejo, Taficillo (Selva 950 m), 26°43'S, 65°17'W, 29.X.2003, fr., *Albornoz et al. 36.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 37.* — *Ibid.*, 29.X.2003, fr., *Albornoz et al. 38.*

Fragaria vesca **L.**
Argentina. Prov. Tucumán, Villa Nougués, 1310 m, 26°51'S, 65°22'W, 16.VIII.1999, fl., *Albornoz 3.* — *Ibid.*, 16.VIII.1999, fl., *Albornoz 4.* — *Ibid.*, 22.XII.1996, fl., *Arias et al. 521.* — Tafí Viejo, Taficillo, Pastizal 1850 m, 26°41'S, 65°20'W, 17.VIII.1998, fl., *Arias et al. 520.* — *Ibid.*, 17.VIII.1998, fl., *Arias et al. 52.* — Tafí Viejo, Taficillo, Pastizal 1870 m, 26°41'S, 65°20'W, 29.X.2003, fl., fr., *Albornoz et al. 25.* — *Ibid.*, 29.X.2003, fl., fr., *Albornoz et al. 26.*

Potentilla tucumanensis **Castagnaro & Arias**
Argentina. Prov. Tucumán, Trancas, Potrero, 1500 m, 26°26'S, 65°33'W, 25.XI.1999, fl., *Albornoz 6.* — *Ibid.*, 25.XI.1999, fl. *Kirschbaum 11* (holotype). — *Ibid.*, 1.X.2003, fl., *Albornoz et al. 10.* — *Ibid.*, 1.X.2003, fl., *Albornoz et al. 11.*