Agrociencia Uruguay 2022 | Volume 26 | Number NE2 | Article 1076
DOI: 10.31285/AGRO.26.1076
ISSN 2730-5066

25 years in 25 articles

Biological characteristics of the promissory forage native legume *Trifolium polymorphum* Poir. (Fabaceae, Faboideae)

Características biológicas de la leguminosa nativa promisoria forrajera *Trifolium polymorphum* Poir. (Fabaceae, Faboideae)

Speroni, G.[1]; Izaguirre, P.[1]

[1] *Universidad de la República, Facultad de Agronomía, Departamento de Biología Vegetal, Montevideo, Uruguay*

Article originally published in:

Agrociencia (Uruguay). 2003;7(1):68-76.
doi:10.31285/AGRO.07.1010.

Correspondence

G. Speroni,
speronig@fagro.edu.uy

Abstract

The biological characterization of *Trifolium polymorphum* Poir., a promissory native forage is presented and taken as a model for its amphicarpy between both reproductive modes. Its esteemed productive and biological characteristics, as well as the good adaptation to climatic and edaphic conditions of the region, high persistency in the sward and plasticity, are shown; despite its low vegetative production, the response to phosphoric fertilization is significantly high.

Biological, organological, ecological, and productive aspects are also described and discussed. The first of them derived from the reproductive double strategy which develops into high subterranean autogamic seed production, leading to genetical homogeneity of their offspring and the lower aerial seed production with a high impact on population dispersion.

Keywords: trifolium polymorphum, amphicarpy, forage, pasture, reproductive biology

Resumen

Se presenta la caracterización biológica de *Trifolium polymorphum* Poir., promisoria forrajera nativa, con características productivas y biológicas valiosas como modelo por su anficarpia entre ambos modos reproductivos. Tiene buena adaptación a las condiciones climáticas y adáficas de la región, alta persistencia en el tapiz, gran plasticidad y, aunque es baja la producción vegetativa, responde en forma significativa a la fertilización con fósforo. Se describen y discuten aspectos biológicos, organológicos, ecológicos y productivos. Los primeros derivados de la doble estrategia reproductiva, que tiene como consecuencia la abundante producción de semillas subterráneas en forma autógama y conduce a la homogeneidad genética de sus descendientes y la menor producción de semillas aéreas, pero con fuerte impacto en la dispersión de las poblaciones.

Palabras clave: anficarpia, forrajera, pasturas, biología reproductiva, trifolium polymorphum

1. Introduction

Trifolium polymorphum ("pink clover", "field carnation", "field clover", "native clover", "polymorphous clover") is a native field component, whose natural range covers Uruguay, the virgin Pampa steppe in the Provinces of Buenos Aires, Entre Ríos, Córdoba, Chaco, Santa Fe (Burkart 1987, Izaguirre, 2001) and in the Provinces of Corrientes, La Pampa, Río Negro and Misiones (Argentina) (Rossow, 1999), the State of Rio Grande do Sul (Brazil), Paraguay and central Chile (Burkart 1987, Izaguirre, 2001). It has been collected in almost the entire territory of our country, being found both in low places with high moisture content in the soil and high and stony places with low water availability. This species is very well adapted to the grassland vegetation and also to the herbivores that inhabit it. These characteristics, added to the good quality of forage production and the excellent palatability, position it as one of the native species regarded as promising forages.

Its amphicarpic condition (production of aerial and underground fruits on the same plant) makes it an interesting object of study from the point of view of reproductive biology. For that matter, studies have started in the Botany Laboratory of the Agronomy College, in order to interpret the biological, ecological, and evolutionary significance of amphicarpy as a life strategy of a perennial legume, adapted to grassland vegetation, given the development of a management plan for the species in natural field, as well as its domestication and subsequent improvement for fodder purposes.

The present study aims to collect information regarding the biological (life cycle, mode of reproduction, pollination, seed production), organological, ecological, and productive characteristics of the species, which are described below.

Growth habit and life cycle

Fifteen species of the genus *Trifolium* (Izaguirre, 1995) have been cited for Uruguay, 12 of them cultivated and subspontaneous and three native: *T. polymorphum* with its variety *grandiflorum*, *T. argentinense,* and *T. riograndense* with its variety *pseudocalyculatum*.

T. polymorphum is a perennial stoloniferous species, with napiform and reserving roots, rooting extant stems up to 40 cm long and 5 to 15 cm tall (Izaguirre & Beyhaut, 1998) (Fig.1, a). The trifoliate leaves have leaflets up to 1.5 cm long and look subglabrous to very hairy, even woolly. Collections carried out in Uruguayan territory allow observing a great variability within the species in terms of the plant vegetative development and the indumentum characteristics.

Due to the habit of stoloniferous growth, it colonizes important surfaces in low covers but needs grazing for its survival since it is eliminated by competition with high pastures (Rosengurtt, 1943, 1946).

It begins to vegetate in March-April, representing an important forage component in the winter months. At the end of flowering, approximately in December, its aerial part disappears during the summer, with the thick reserve root remaining underground.

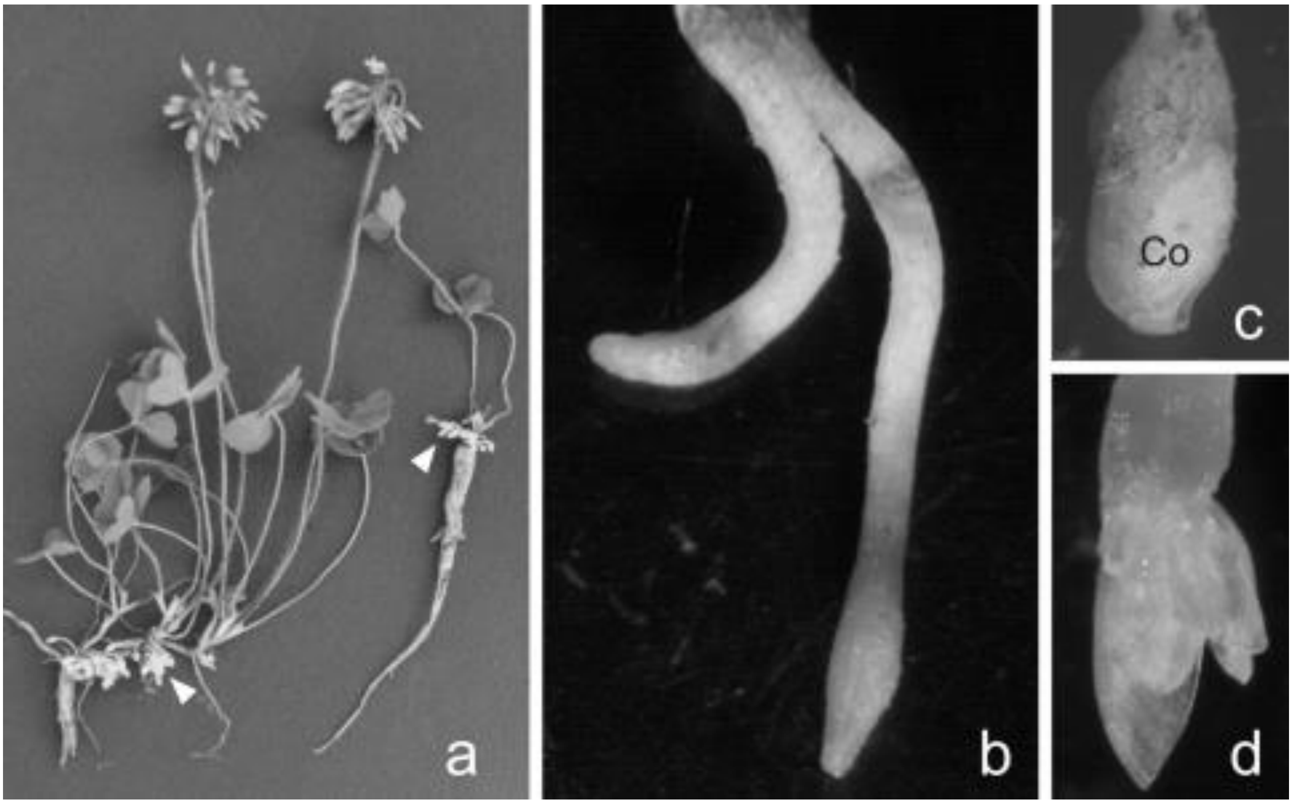
Organological and biological characteristics

T. polymorphum is an amphicarpic species (from the Greek ***anfi***, both; ***carpo***, fruit), that is, it produces two types of fruits, aerial and underground, derived respectively from two different types of flowers (Fig. 1, a). There are about thirty amphicarpic species in the world (Fevereiro-Barbosa, 1987), and several belong to the genera of legumes such as *Vicia*, *Amphicarpa*, *Vigna*, *Trifolium* (Rivals, 1953), and *Macroptilium* (Fevereiro-Barbosa, 1987). Within the native legumes, two amphicarpic species belonging to the *Trifolium* genus have been recognized: *T. polymorphum* and *T. argentinense*, but citations can increase if this aspect is taken into account in future plant collections.

The aerial flowers of *T. polymorphum* are pink, with papilionaceous corolla, grouped in long pedunculated heads and are usually produced from October to November. They are chasmogamous and bear fruit until December, developing ovoid to elongated legumes, 2-4 seeded.

The underground flowers are produced in positively geotropic bundles, inserted in the knots of the runners, from the winter months onward. They present marked morphological modifications compared to the floral typology of the aerial ones (Speroni, 2000), which result from the adaptation to underground life. They are colorless flowers, with thickened cap-shaped calyx, which protects the most delicate internal whorls during soil penetration (Fig. 1, b). The corolla resembles the papilionaceous type of aerial flowers, but with a reduction in number to three pieces and evident color loss. The larger external petal resembles the standard of papilionaceous corolla, while the two symmetrical inner petals are homologous to the wings of this type of corollas (Fig. 1, d). The inner petals, that form the keel on the papilionaceous corolla, have disappeared. The protection function offered by the keel to fertile whorls and the role it plays as a guiding piece and landing platform for floral visitors ceases to make sense in an underground flower where the protection of the flower is achieved by more resistant pieces such as the calyx, and the activity of pollinators is evidently not necessary. The number of stamens is also reduced to three, which develop achieving intimate contact between anthers and stigma. The underground fruit is a globose legume that houses two seeds that ripen from September to December.

Figure 1. *Trifolium polymorphum*. a) Plant with aerial and underground flowers (arrows). b) Detail of two underground flowers with the calyx completely covering the flower. c) Underground flower with visible corolla (Co). d) Underground flower with removed calyx, showing the three corolla petals.



Mode of Reproduction

Within the legume family, Papilionoideae are reported as predominantly self-compatible and herbaceous (Arroyo, 1981; Shivanna and Owen, 1989). However, the northern hemisphere species belonging to the Trifolieae tribe, have a higher percentage of self-incompatibility than other

herbaceous tribes (Arroyo, 1981). Within the *Trifolium* genus, self-incompatible and self-compatible species have been cited and according to data presented by Arroyo (1981), out of 28 species of this genus studied, 17 were self-incompatible (60.71%).

The aerial flowers of *T. polymorphum* are chasmogamous (from the Greek ***catmo***, opening; ***gamia***, junction) and some of their characteristics are common to insect-pollinated flowers. The papilionaceous corolla of bilateral symmetry, coloration in different shades of pink, which intensifies towards the base of the petals, the intense perfume, and the presence of a nectary at the ovary base, are characteristics that capture insects' attention and guide them to the reward offered by the flowers to their potential pollinators: nectar. These flowers were referenced as allogamous (Coll and Zarza, 1992); however, there is poor information on the collection of these data. Research on the reproductive biology of *T. polymorphum* (Speroni, 2000) in a population located in the Department of Lavalleja (Uruguay), the stigma was observed to present esterase activity from early stages of floral development and it remains receptive until the anthesis or opening of the flower. Anthers dehiscence occurs in these cases before the anthesis of the flower, therefore the pollen, which contains the precursor cell of the male gametes, can reach the stigma of the same flower, without the need to transport to other flowers. In the studies of embryo sac development and female gamete formation with serial cuts at the ovarian level, zygote formation was observed before flower anthesis, in some sectioned ovules (Speroni, 2000; Speroni and Izaguirre, 2001). Seed production was also recorded in heads that were isolated with paper bags to prevent the arrival of foreign pollen (Speroni, 2000). All these data allow concluding that there are autogamy processes in this studied population, but it is necessary to extend this type of study to different populations of the species and analyze the biology of the flower in more detail.

The underground flowers are cleistogamous (from the Greek ***cleitto***, closed; ***gamia***, union), that is, the pollination and consequent fertilization, occur with the flower closed and therefore the produced seeds are the result of an autogamy process. In this regard, according to Uphof (1938), there are species with ecological cleistogamy, where the production of cleistogamous or chasmogamous flowers is conditioned by ecological factors. These are species where climatic conditions, soil conditions, nutrient availability, etc., affect the reproductive mode of flowers. But in other cases, cleistogamy does not depend on external factors but inherited factors. Such is the case of amphicarpic species, where aerial chasmogamous and underground cleistogamous flowers are formed, which present notorious modifications in morphology, according to Uphof (1938), as described in previous paragraphs for *T. polymorphum*.

Studies on the reproductive biology of these flowers (Speroni, 2000; Speroni and Izaguirre, 2001) confirmed the processes of autogamy and detected functional modifications that favor it, such as the absence of dehiscence of the anthers and the germination of the pollen tubes crossing the anther wall towards its stigma. Although the wall of the pollen sac has endothetium, it seems to have lost its functionality for the opening of the anther.

The possibility of development of apomictic embryo sacs was ruled out, as no evidence has been found so far in embryological studies.

Pollination

Papillionoids constitute a subfamily pollinated mainly by hymenoptera, which are considered very important in its evolution (Leppik, 1966). Within *Trifolium*, bees have been found as pollinators, as well as bumblebees of the genus *Bombus* (especially in the northern hemisphere) and *Xylocopa* (Arroyo, 1981).

In the systematic observations carried out to register type and activity of floral visitors, and their possible role as pollinators in the aerial flowers of a *T. polymorphum* population in the Department of Lavalleja (Speroni, 2000), the insects that are detailed in Table 1 were found. They were mainly beetles that used the head as a shelter and food source, therefore being extremely destructive to the plant. While they could touch fertile whorls by eating flowers, the energy cost to the flower is very high. If considering beetles as possible pollinators, they are insects that would favor autogamy or geitonogamy, since they usually stay on the same flower for a long time or their movements are very short, remaining inside the head. The flight to other heads was observed on very few occasions.

Table 1. Floral visitors observed in aerial flowers of *Trifolium polymorphum* from a population located between Del Cuervo and Arequita hills (Lavalleja Department). Systematic observations of 10 minutes were performed on 3 patches at the times: 7:00, 9:00, 11:00, 13:00, 15:00, 17:00, 19:00 and 21:00 h, for 2 days.

Order	Family	Species
Beetle	Chrysomelidae Chrysomelidae	*Temnodachrys* sp not identified
	Curculionidae	not identified
Lepidoptera		not identified

The morphology of the aerial flowers of *T. polymorphum* seems adapted to bee pollination: bilateral symmetry, orienting the insect arrival; the most intense coloration towards the base of the petals reinforcing the signal of the flower and indicating the location of the floral reward; and perfume, acting as a floral attraction. While (*Apis mellifera*) bees have been observed visiting *T. polymorphum* flowers in some wild populations, in field tests where plants of *T. repens* and *T. pratense* were also present, bees only visited these last two species.

Research on pollination has placed special emphasis on floral adaptations regarding the pollinator (floral syndromes). However, Frankel and Galum

(1977) cite species such as *Vicia sativa*, *Medicago hispida* and *Trifolium fragiferum* which are autogamous, although there is an adaptation to entomophilia. In recent years, studies on pollination have tended to show that specialization is not the most common in pollination systems and that it is difficult to predict the type of pollinator through floral syndromes (Waser and others, 1996; Herrera, 1996). Frankel and Galum (1977) suggest that structural adaptations to allogamy can often be evolutionary remnants in species that acquire a new reproductive system. Herrera (1996) points out that the floral characteristics that a species currently presents may have evolved with respect to other aspects of its reproductive biology and not necessarily in relation to its current pollinator. In these cases, floral morphology would be more an exaptation than an adaptation.

Many self-compatible legumes have a mechanical pollination system called "tripping", which consists of the presence of a stigmatic cuticle that prevents the early germination of pollen grains on the stigma of the same flower. When this cuticle breaks due to a mechanical impact (usually by a visiting pollinator), it allows the pollen to adhere to the stigma and germinate, and the visitor also brings, in its body, pollen from other flowers it has visited. This is a mechanical way to promote cross-pollination in self-compatible species. The genus *Trifolium* is characterized by presenting valve-type tripping (Leppik, 1966), where stamens and stigma emerge from the keel due to the

visitor's pressure and then return to the initial position, therefore, these flowers need repeated visits. In *T. repens* and *T. pratense* species, the stigmatic papillae are covered by a cuticle, below which a lipid secretion is found. When a pollinator breaks the cuticle, this lipid secretion is released favoring the adhesion of pollen grains (Leduc and others, 1990; Heslop-Harrison and Heslop-Harrison, 1983). Germination was verified at the stigma level when observing *T. polymorphum* pollen tubes in a fluorescence microscope. This indicates that there may be a stigmatic structure similar to that observed in *T. repens* and *T. pratense* and that, although it is self-compatible, it also has a "tripping" mechanical barrier that favors cross-fertilization.

Field observations need to be extended to more populations of *T. polymorphum* to elucidate the role of floral visitors in the reproductive system of the species.

Seed production

Seed production in the aerial and underground flowers of *T. polymorphum* is very different numerically. Although quantification and statistical analyses have not been carried out yet, the production of underground seeds seems to be noticeably higher than the production of aerial seeds, generating important seed banks in the soil.

Extrinsic and intrinsic factors have been detected, among the causes of the low production of aerial seeds. Within the extrinsic, herbivory is one of them. As noted by Rosengurtt (1943), cattle like eating the inflorescences. Field studies (Speroni, 2000) have also detected the herbivory of insects, especially beetles, which feed on the floral parts and even oviпose inside the flower ovary and their larvae feed on the seminal primordia. Among the intrinsic causes, alterations were detected in the meiotic processes of microspores formation, reducing the number of pollen grains that reach complete development in the pollen sacs (Speroni, 2000). Likewise, pollen viability tests, using the fluorochromatic reaction (FCR) developed by Heslop-Harrison (1970, in Shivanna and Ragaswamy, 1992) with fluorescein diacetate (FDA) as a dye, presented highly variable values (34% ± 23) (Table 2).

The ovary of the underground flower houses 2 eggs (fewer than the aerial ones) and all of them usually develop successfully as seeds. In addition, the underground flowers are not subject to predatory pressure, since no flowers, fruits, or seeds were found to present significant symptoms of it (Speroni, 2000). These flowers develop in an environment with more constant conditions, which allows greater stability for the processes of sporogenesis, gametogenesis, fertilization and embryonic development (Plitman, 1973). It also allows the inoculation of seeds produced with *Rhizobium* strains genetically adapted to the genotype, soil, and environment where they develop

All these characteristics favor the development and production of underground seeds, generating a fast and safe reseeding in the same place as the mother plant. But if seeds are considered one of the most important evolutionary novelties of terrestrial plants, which allow the dispersion of the species by colonizing new environments, avoiding the attack of natural enemies (frequent in the vicinity of the mother plant) and restricting competition between offspring during germination (Willson, 1992), the underground seeds of *T. polymorphum* would be in a disadvantaged position for having very restricted dispersion and high competition of the offspring during germination. On the other hand, the more stable conditions of the soil around them would be highlighted as an advantage, with fewer physical (absence of photoperiod, constant temperatures, etc.) and chemical alterations (no passage through the rumen and digestive system of herbivores) that cause a longer latency than other legumes. Thus, the germination of these seeds could occur in a more staggered way, avoiding in part, the competition between siblings.

Table 2. Viability test of aerial flower pollen grains using FCR and FDA as dye. Samples were observed under Olympus Vanox AH-3 fluorescence microscope, with filter for the blue excitation range (B). Each sample corresponds to a flower.

Sample	Counted Grains	Viable		Non-Viable	
1	505	308	60.99%	197	39.01%
2	519	424	81.70%	95	18.30%
3	219	57	26.03%	162	73.97%
4	418	184	44.02%	234	55.98%
5	423	46	10.87%	377	89.13%
6	787	86	10.93%	701	89.07%
7	363	204	56.20%	159	43.80%
8	289	171	59.17%	118	40.83%
9	270	69	25.56%	201	74.44%
10	241	74	30.71%	167	69.29%
11	168	11	6.55%	157	93.45%
12	373	33	8.85%	340	91.15%
13	249	45	18.07%	204	81.93%
14	684	283	41.37%	401	58.63%
Total					

Mean viability: 34.36%
SD: 23.43

Ecological considerations

T. polymorphum is a grassland species and its vegetative characteristics and life strategy are a clear reflection of adaptation to this type of vegetation and the herbivores that inhabit it. Being a stoloniferous species, long-lived perennial due to its reserve roots, the plants persist vegetatively year after year, even without the need to regenerate by seeds. The renewal meristems of vegetative organs, located in the stolons, are free of the harvest horizon of ruminants and other herbivores, favoring persistence during vegetative development.

Since *T. polymorphum* presents horizontal development of the stoloniferous stems and does not grow tall, it disappears due to competition with tall grassland species that are eliminated by grazing. This greatly favors its permanence in the herbaceous cover.

This adds to the amphicarpic condition of the species, which creates important seed banks in the soil by producing underground seeds, which are not significantly affected by the fairly frequent climatic variations of the different years.

Adaptation of grasses to grassland conditions and their coevolution with herbivores has already been proven. This legume behaves in accordance since it has other biological characteristics that position it as one of the best equipped to thrive in the ecosystem it inhabits.

Agronomic characteristics

T. polymorphum is an important member of natural grasslands, both for its good adaptation and its quality and palatability. In winter it represents an important forage component, especially for wool cattle, due to its low size, in unbroken, usually fertilized, soils. Although it presents low vegetative production, analyses carried out by the Nutrition Laboratory of INIA reveal protein values of 22% (Coll and Zarza, 1992). Studies carried out in the Province of Corrientes (Argentina) found that it can reach 10% coverage in grazed fields, and it can rise from 30 to 60% when the land is fertilized with phosphorus (Fernández and others, 1988). In Uruguay, natural field paddocks fertilized with phosphorus and kept very low during the summer, present from autumn onwards, pastures with a high proportion of *T. polymorphum* (Coll and Zarza, 1992).

It is a legume of great adaptability and plasticity to different soil conditions: humiferous and moist from flooded mountain plains with pH 7; fertile and dry with pH 5; deep and very fertile and permeable humiferous with pH 6.5; poor and whitish, waterproof, scarce in organic matter with pH 4.5; clayey, waterproof and dry with pH 5; as well as sandy and permeable, plain and high with pH 4.75 (Izaguirre, 2001).

This characteristic makes it different from the other amphicarpic species, *T. argentinense*, which is restricted to uliginous soils, even flooded and does not overcome grassland transformations (Burkart, 1952), for which it has not yet been the subject of special studies.

Climatic characteristics of its natural range, with periods of abundant rainfall and also long drought periods, and average temperatures between 15 and 25 °C, seem to be ideal for its development.

The good production of underground seeds and the accumulation of root reserves have allowed the species to survive even in soils with high risk of drought and with continuous, intense, and selective sheep grazing (Coll and Zarza, 1992).

The high persistence in the field, the good quality of its fodder, the wide distribution in our territory, and the special season of its vegetative production when other forages are scarce, are highly valued when evaluating forage species (Coll and Zarza, 1992).

Final considerations

The species *T. polymorphum* constitutes an interesting object of study, both for its economic potential as a promising native forage, as well as for the biological characteristics it possesses that justify its study as a source of basic knowledge to interpret the biology of reproduction in plants. The amphicarpic condition with aerial flowers where autogamy processes and low production of seeds were detected and autogamous underground flowers with abundant seed production in the same place as the mother plant, generates a population structure that tends to its genetic homogeneity. The production of aerial seeds, although in low quantity, autogamous, and/or eventually allogamous, can have an important impact on the genetic mobility of the species, both by the dispersion of its seeds and by the mode of pollination of the flowers. The offspring from allogeneic crossings introduce variability in the populations and ensure persistence over time, due to the adaptability achieved by new genotypes. Seed production in aerial flowers is important for populations in the long term, to maintain genetic diversity and the ability to adapt to environmental changes.

Based on the studies carried out, several questions remain about this species: are aerial flowers predominantly autogamous or allogamous?, do they need an effective pollinator?, can we think of different modes of reproduction for the two types of flowers?, can the plant regulate two different reproductive types?, what is the genetic structure of the populations? to answer these questions, it is necessary to continue studying the biology of *T. polymorphum*, understand its variability and characterize one of the promising species of our flora.

Acknowledgments

To the Basic Sciences Development Program (PEDECIBA by its Spanish acronym) of the Universidad de la República, framework for the Thesis Project of the first of the authors' Master in Biology, Botanical Option. To the Sectoral Commission for Scientific Research (CSIC by its Spanish acronym) for the financial support granted for the completion of this thesis. To Adj. Prof. Carlos Bentancour, from the Plant Production Department of the Agronomy College of Montevideo, for the identification of floral visitors.

References

ARROYO, M. T. 1981. Breeding systems and pollination biology in Leguminosae. In: Polhill, R. & P. Raven (Eds.). Advances in Legume Systematics. 2: 723-769. Roy. Bot. Gard., Kew.

BURKART, A. 1952. Las Leguminosas argentinas silvestres y cultivadas. Ed. ACME. Buenos Aires. 569 pp.

BURKART, A. 1987. Flora Ilustrada de Entre Ríos. III. Colección del INTA. 6. Buenos Aires.

COLL, J. y A. ZARZA. 1992. Leguminosas nativas promisorias. Trébol polimorfo y babosita. Boletín de Divulgación N° 22. Instituto Nacional de Investigación Agropecuaria (INIA). Uruguay.

FERNÁNDEZ, J. G., C. A. BENITEZ, R. M. PICIO y O. R. PALLARES. 1988. Leguminosas Forrajeras Nativas del Este de la Provincia de Corrientes. Serie Técnica N° 26. INTA. Argentina.

FEVEREIRO-BARBOSA, V.P. 1987. Macroptilium (Bentham) Urban do Brasil (Leguminosae-Faboideae-Phaseoleae- Phaseolinae). Arq. Jard. Bot. Rio de Janeiro 23: 109-180.

FRANKEL, R. y E. GALUM. 1977. Pollination Mechanisms, Reproduction and Plant Breeding. Springer-Verlag.281 pp.

HERRERA, C. M. 1996. Floral Traits and Plant Adaptation to Insect Pollinators: A Devil's Advocate Approach. In: Lloyd, D. G. & S. C. H. Barrett (Eds.). Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants.:65-87. Chapman & Hall.

HESLOP-HARRISON, J. y Y. HESLOP-HARRISON. 1983. Pollen-stigma interactions in the Leguminosae: the organization of the stigma in Trifolium pratense L. Ann. Bot. 51: 571-583.

IZAGUIRRE, P. 1995. Especies indígenas y subespontáneas del género Trifolium L. (Leguminosae) en el Uruguay. Serie Técnica N° 58, Instituto Nacional de Investigación Agropecuaria (INIA). Uruguay.

IZAGUIRRE, P. 2001. Trifolium polymorphum Poir. In: Grassland and Pasture Crops. http://www.fao.org/ WAICENT/FAOINFO/AGRICULT/AGP/AGPC/doc/ pasture/pasture.htm

IZAGUIRRE, P. y R. BEYHAUT. 1998. Las Leguminosas en Uruguay y regiones vecinas. Parte 1. Papilionoideae. Ed. Hemisferio Sur. Uruguay.

LEDUC, N., G.C. DOUGLAS, M. MONNIER y V. CONNOLLY. 1990. Pollination in vitro: effects on the growth of pollen tubes, seed set and gametophytic self-incompatibility in Trifolium pratense L. and T. repens L. Theor. Appl. Genet. 80: 657-664.

LEPPIK, E. E. 1966. Floral evolution and pollination in Leguminosae. Ann. Bot. Fennici 3: 299-308.

PLITMAN, U. 1973. Biological flora of Israel 4. Vicia sativa ssp. amphicarpa (Dorh.) Aschers. & Graebn. Israel J. Bot. 22: 178-194.

RIVALS, P. 1953. Sur quelques Légumineuses géocarpes et amphicarpes. Rev. Int. de Bot. Appl. 33: 244-249.

ROSENGURTT, B. 1943. Estudios sobre praderas naturales del Uruguay. 3ª Contribución. La estructura y el pastoreo de las praderas en la región de Palleros. Flora de Palleros. Barreiro y Ramos S.A. Uruguay.

ROSENGURTT, B. 1946. Estudios sobre praderas naturales del Uruguay. 5ª Contribución. Normas usuales de manejo de praderas naturales en J. Jackson. Flora de J. Jackson. Imprenta Rosgal. Uruguay.

ROSSOW, R. A. 1999. Trifolium L. In: F. O. ZULOAGA y O. MORRONE (Eds). Catálogo de las Plantas Vasculares de la República Argentina II. Fabaceae-Zygophyllaceae (Dicotyledoneae). Missouri Botanical Garden Press: 734-737.

SHIVANNA, K. R. y S. J. OWENS. 1989. Pollen-pistil interactions (Papilionoideae). In: C.H. Stirton y J.L. Zarucchi (Eds.). Advances in Legume Biology. Monogr. Syst. Bot. Missouri Bot. Gard. 29: 157-182.

SHIVANNA, K. R. y N. S. RANGASWAMY. 1992. Pollen Biology. A Laboratory Manual. Springer-Verlag. 119 pp.

SPERONI, G. 2000. Aspectos de la Biología Reproductiva de Trifolium polymorphum Poir. (Fabaceae, Papilionoideae). Tesis de Maestría en Ciencias Biológicas. Opción Botánica. Programa de Desarrollo en Ciencias Básicas. Uruguay.

SPERONI, G. y P. IZAGUIRRE. 2001. Morfología y Esporogénesis en Flores Aéreas y Subterráneas de la Especie Anficárpica Trifolium polymorphum (Fabaceae, Papilionoideae). Bol. Soc. Argent. Bot. 36 (3-4): 253-265.

UPHOF, J. C. TH. 1938. Cleistogamic flowers. Bot. Rev. 4: 21- 49.

WASER, N. M., L. CHITTKA, M. V. PRICE, N. M. WILLIAMS y J. OLLERTON. 1996. Generalization in pollination systems and why it matters. Ecology 77 (4): 1043-1060.

WILLSON, M. F. 1992. The Ecology of Seed Dispersal. In: Fenner, M. (Ed.). Seeds. The Ecology of Regeneration in Plant Communities. :61-85. CAB International.