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Studies of rust fungi on *Lotus subbiflorus* and *L.uliginosus*

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Introduction

Root and crown diseases reduce persistency of legumes, whereas foliar diseases usually interfere with the normal functions of the leaf. Sometimes they cause defoliation, which reduces yield and quality of forage. Altier (1997) has done an extensive research in diseases of *Lotus corniculatus* L. in Uruguay. The survey demonstrates that the incidence and severity of leaf diseases vary with the seasons and climatic conditions. The most prevalent diseases (*Phoma*, *Colletotrichum*, *Phomopsis* and *Cercospora)* developed mostly in spring, whereas rust caused by *Uromyces* spp. can be found from February to April.

L.corniculatus has been the predominant legume in Uruguayan pastures for the last four decades. Most of the locally adapted cultivars (San Gabriel, Estanzuela Ganador, INIA Draco) have good resistance to *Uromyces*, suggesting that the breeding program selected against this trait. The area of the annual *Lotus subbiflorus* has increased greatly during the last decade in Uruguay, expanding to adjacent regions of Brazil and Argentina. More recently, *Lotus uliginosus* became another species with great potential for rangelands. Foliar diseases on *L. subbiflorus* and *L. uliginosus* have not been extensively studied.

In early November 2000, rust was observed on *L. subbiflorus* at Palo a Pique Experimental Field, Treinta y Tres, Uruguay (V.Olivieri, INIA internal report, 2001). Rust severity ranged from trace to moderate at several other plot sites within a 90 km radius. Only the uredinial stage of the fungus has been observed. Uredinia were mostly found on leaflets, but petioles and stems were also infected. The following year (2001) rust was prevalent in epidemic proportions on seed production plots, where all the lower leaves were killed and stem infections caused the distal parts to wilt and die. The disease may appear at any time during the growing season when warm and wet weather prevails, but it develops most abundantly at seedling stage in early autumn and at flowering stage in late spring.

Rust was also recorded on *L. uliginosus* cv. Grassland Maku in 2001, when severity ranged from trace to low. The disease severely damaged spaced plants the following year (2002) at La Estanzuela, Colonia. Uredinia were observed in leaflets and stems, which turned yellow and drop or dried off**.** Maximum damage was observed during dry spells, when vegetative growth was stunted and pustules developed up to the top leaflets. Neither the presence of telia has been verified on *L. uliginosus* nor *L. subbiflorus*.

Field observations of the pathogen have been reported on several *Lotus* species worldwide as well as in the region, among other forage legume species**.** Zeider (1985) reported rust of epidemic proportion on *L. corniculatus* in USA and *Uromyces striatus f. medicaginis* has been reported as a miscellaneous disease on *L. subbiflorus* in Hawaii (Gardner, 1994). Regional reports of the disease refer to *U.loti* (*U. euphorbiae-corniculati*) on *Lotus glaber* in Argentina (Juan *et al.,* 2000) and on *L. corniculatus* in Uruguay (Altier, 1997).

The literature quotes rust on the genera *Lotus* to belong to a number of species and/or physiological races which differ in their ability to attack different host genera and species and different varieties within a species. Arthur (1934) identified two species of rust (*U.striatus* loti and *U. punctatus*) collected on the alternative host *Euphorbia cyparissias* as the pathogen that causes the disease on *L. corniculatus*. In addition to this ornamental plant, aecia and pycnia are produced on some congeneric species in Europe. The occurrence of 6 species of *Uromyces* in the tribe Loteae is reported by El-Gazzar (1981). Broad-spectrum species of the disease included *U. anthyllidis, U. striatus, U. pisi* and *U. genistae-tinctoriae,* reported in 7 Tribes in addition to Loteae, whereas *U. euphorbiae-corniculati* is reported on *Vicia* and *Lotus*. On the opposite, *U. loti* is specific of the genera *Lotus* and *Tetragonolobus*.

In addition to the importance of the rust outbreak on two *Lotus* species in Uruguay, rust has developed on cultivated soybean in some regions of the Southern Cone. The lack of information on the host range of the disease in the country determined the need to begin a series of greenhouse test to identify the rust species/races. A differential set with different *Lotus* species was designed for this purpose by the forage research group and the Cereal Disease Laboratory at INIA La Estanzuela in 2002. The present work compared cross-inoculations of rust collected in either cultivated or endemic species of *Lotus* in Uruguay, aimed to identify the host range in cultivated forage legume species.

Materials and Methods

Urediniospores from *L. uliginosus* cv Grassland Maku (Strain 1) and *L. subbiflorus* cv El Rincón (Strain 2) were collected at La Estanzuela, Colonia and La Carolina, Flores, respectively. Strain 3 was harvested from spontaneous plants of *L. corniculatus* in the surroundings of La Estanzuela. Inoculation tests were carried out on *L. corniculatus* cv. San Gabriel, *L. uliginosus* cv. G.Maku, *L. subbiflorus* cv. El Rincón, *Medicago sativa* cv Estanzuela Chaná, *Trifolium pratense* cv Estanzuela 116 and *Trifolium repens* cv Estanzuela Zapicán (Table 1).

Rust collection was done with a cyclone spore collector (Cherry and Peer, 1966) and urediniospores were vacuum dried and stored under refrigeration (4°C). Pots containing 8-10 seedlings at the stage of 5-8 leaflets were inoculated by spraying with a urediospores suspension in Soltrol 170 (Phillips Petroleim, Bartlesvill, OK) mineral oil, a technique used for inoculations of wheat rust in greenhouse tests (Rowell, 1984). A total of 9 replications per species were evaluated. In addition, one pot of soybean was inoculated with each strain.

Plants were located in a humid and dark chamber during 24 hours after inoculation. First symptoms of the disease appeared approximately 10 days after inoculation and rust rating was scored in the most severely infected leaflet on each plant 14 days after inoculation. The scale follows the one described by Skinner and Stuteville (1995): resistant (no symptoms), moderately resistant (flecks and closed pustules), moderately susceptible (closed pustules and small open pustules), susceptible (small open pustules), highly susceptible (medium to large open pustules).

Results and Discussion

Common varieties of rust that attack clovers cannot be distinguished on the basis of symptoms but can be differentiated by their capacity to infect the various legumes. The present study suggests that all three strains were specific to the genera *Lotus,* since neither infected the cultivars of *Medicago sativa, Trifolium repens* or *T. pratense* tested (Table 1). This specificity is supported by research developed by Zeiders (1985) in USA, who mentions *Uromyces striatus* var. *loti* as the possible causal fungi causing *Lotus* leaf rust. Furthermore, Skinner and Stuteville (1995) and El-Gazzar (1981) reported strains of *U. striatus*, found as pathogens of alfalfa, being capable of surviving and reproducing on a broad range of plant species (including Trifolieae and Vicieae), but no susceptibility was found in the tribe Loteae *(L. corniculatus, L. uliginosus, L. angustissimus*) by Skinner and Stuteville (1995).

Table 1. Cross-inoculation reaction of rust strains.

	Strain 1	**Strain 2**	**Strain 3**
Lotus uliginosus	++	+	-
Lotus angustissimus	+	-	-
Lotus subbiflorus	-	++	-
Lotus corniculatus	-	-	++
Medicago sativa	-	-	-
Trifolium pratense	-	-	-
Trifolium repens	-	-	-

++ highly susceptible
+ moderatelly susceptible
- resistant

Rust on *L. uliginosus* (Strain 1) infects *L. uliginosus* (Figure 1) and *L. angustissimus.* The rust on *L. subbiflorus* (Strain 2) infects also two species (*L. subbiflorus* – Figure 2 and *L. uliginosus* –Figure 3), whereas Strain 3, collected on *L. corniculatus*, was specific for the species (Figure 4). No references about cross-inoculation within the genera *Lotus* have been found in the literature to support these results. However, *Medicago* and *Trifolium* rusts are good examples of case studies where one strain could infect several species of the same genera (Skinner and Stuteville, 1995; Hanson and Kreitlow, 1953). Reactions of Strain 1 and Strain 2 were more susceptible in the original species where the samples were collected than in the

other host species, which had moderately susceptible reactions (Figures 1 and 3). Rust strain mixtures were discarded by single pustule isolations inoculation on cross species.

Figure 1. Strain 1 on underside of *L. uliginosus* leaflets.

Figure 2. Strain 2 on upperside of *L. subbiflorus* leaflets.

Figure 3. Strain 2 on upperside of *L. uliginosus* leaflets.

Figure 4. Strain 3 on underside of *L. corniculatus* leaflets.

Symptoms on *L. subbiflorus* are usually limited to the leaflets in the greenhouse inoculations, although they may occur anywhere on petioles and stems. The earliest symptom is the development of minute, light-yellow spots, mostly on the upperside leaflet surface, similar to the early symptoms of other leaf spot diseases. Spots enlarge and become pustules, mostly oval, developing on either leaflet surface. Single fully developed pustules measure about 0.5-1 mm across, although they are sometimes arranged in circles around a single pustule. When pustules are abundant the entire leaflet may turn yellow, die and fall off. Symptoms on *L. uliginosus* are slightly different. Pustules are seen mainly in the underside of the leaflets and in very severe cases in stems, under the shape of small circular light brown color pustules that break the epidermis and expose the urediniospores. No telial state has been found in both species.

Phakopsora pachyrhizi was descarted as the causal pathogen, since *Lotus* rust has no paraphyses**,** uredia on both sides of the leaflets and on stems, and urediniospores with thick walls (1.5 to 2 µ), features that do not match with *P. pachhyrhizis* (Ono *et al*, 1992) *Phakospora pachyrhizi.* Furthermore, none of the strains developed symptoms on soybean in the greenhouse.

Isolates used may not represent the whole rust population present in the country. Moreover, the present study has a narrow range of host species and cultivars. For those reasons, these results should be considered as representing the minimum host range that a fungus causing *Lotus* rust may have. Understanding the fungus life cycle and host range will provide an insight of the potential risk of the disease. From an epidemiological standpoint, results suggest that an outbreak of *L. uliginosus* rust may originate on this species or arise from at least two other species (*L. angustissimus* and *L. subbiflorus*). Although the relative importance of these species in the epidemiology of *L. uliginosus* is unknown, it is now clear that they are capable of playing a significant role in innoculum increase and dissemination. There are no reports in the literature that explore the pathogen host range within the genera *Lotus* to support these research findings.

Screening for resistance to Strain 1 has allowed to identify resistant plants in *L. uliginosus*. Further studies should address to a wider pathogen sampling, with isolates from different areas, as well as an increased range of host species and cultivars within species, in order to know the pathogen spectrum.

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