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Epidemiological studies on crown and root rot of birdsfoot trefoil in Uruguay

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Abstract

Birdsfoot trefoil fields in 3 locations in Uruguay were surveyed to determine incidence and severity of crown and root diseases in 1- to 3-yr-old stands. Twenty-five plants in each of 12 permanent quadrats were evaluated at each site (n = 300 plants per site, 3 sites per location). Plants were scored for disease severity following a 5-class scale: 0 = no disease, 4 = dead plant. Crown and root rot occurred in every site, with average incidences (percent infected plants) of 43, 96, and 100% and average severities of 0.51, 1.51, and 1.86 in 1-, 2-, and 3-yr-old stands, respectively. *Fusarium oxysporum* was the primary pathogen associated with diseased plants. Variance to mean ratios for disease severity among quadrats within sites were consistently less than 1, suggesting that disease was not aggregated among quadrats in individual sites. Stand counts decreased with age, from 200 plants/m^2 in 1-yr-old to less than 50 plants/m^2 in 3-yr-old stands. Stand counts also decreased with increasing disease intensities among stands of the same age, suggesting a relationship between crown and root rot and plant persistence. Resistant cultivars and proper utilization practices should be considered as potential means for disease management.

Keywords: birdsfoot trefoil, crown and root rot, *Fusarium oxysporum*, *Lotus corniculatus*, persistence

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is the most important forage legume in Uruguay, where it is used for pasture, hay or silage. In addition, seed production is an important export enterprise. Birdsfoot trefoil is usually undersown with a cereal crop during the fall, and plays an important role in the sustainability of crop-pasture rotations. The major constraint for the use of birdsfoot trefoil is its relatively poor persistence. In Uruguay, significant plant losses are observed in pastures in stands two-years-old and older, especially following periods of environmental stress (i.e. summer drought) or under continuous grazing systems (Formoso, 1993). These stands become unprofitable and the farmer may decide to start a new crop, resulting in a short legume-phase in the rotation. A similar situation is reported by several authors in North America (Beuselinck *et al.*, 1984; Hoveland, 1989; Miller *et al.*, 1964;

Taylor *et al.*, 1973). The limited persistence is generally attributed to the interaction of several abiotic and biotic factors such as climatic and edaphic stresses, diseases and pests, and management practices that produce a cumulative stress load (Grau, 1996; Leath, 1989). The use of birdsfoot trefoil could be increased if highly productive stands could be maintained under intensive management for several years.

Diseases are a major cause of premature stand decline and reduced productivity in most temperate forage legumes (Leath, 1989; Leath *et al.*, 1996). Crown and root diseases have been identified as the most important limitations to birdsfoot trefoil production and persistence (Berkenkamp *et al.*, 1972; Beuselinck, 1988; Drake, 1958; Grant and Marten, 1985; Henson, 1962; Hill and Zeiders, 1987; Hoveland *et al.*, 1982; Hoveland *et al.*, 1987; Kainski, 1960; Miller *et al.*, 1964; Pettit *et al.*, 1966; Taylor *et al.*, 1973). Severe losses from these diseases are usually associated with warm weather and high humidity, and thus these are of greater importance in the South than in the Northeast or Northcentral U.S. (Beuselinck, 1988; Grant and Marten, 1985). However, these diseases have been recently reported in most regions where birdsfoot trefoil is grown (Altier, 1994; Bergstrom *et al.*, 1995; Viands *et al.*, 1994). In Uruguay, Altier (1994, 1997) found that 93% of birdsfoot trefoil plants from a space plant nursery died by the end of the second year, and 82% of plant losses were due to crown and root diseases.

The first symptom of crown and root infection is the failure of the plants to resume growth after harvest (Berkenkamp *et al.*, 1972; Grau, 1996; Henson, 1962; Kainski, 1960). Infected plants have a low tolerance to water stress during summer months and reduced vigor; if the invasion continues, plants become wilted and die. Diseased plants show necrosis and rotting of crown and root cortical tissues, but discoloration may be restricted to the central core and follow the vascular system (Figure 1). As the disease develops, both the cortex and central core may be invaded by the fungus. Necrotic areas are often associated with wounds in the crown or root surface (Altier, 1994; Leath *et al.*, 1971). Insect feeding injury by root curculio probably enhances infection by soil pathogens (Kalb *et al.*, 1994; Leath and Hower, 1993).

Figure 1. Symptoms of crown and root rot in a diseased birdsfoot trefoil plant.

Crown and root diseases are caused by a complex of soil organisms. Although several genera of fungi including *Rhizoctonia*, *Mycoleptodiscus*, *Macrophomina*, *Phoma*, and others have been isolated from diseased plants, *Fusarium* species make up the largest number of pathogens causing crown and root diseases of birdsfoot trefoil (Berkenkamp *et al.*, 1972; Beuselinck, 1988; Drake, 1961; Henson, 1962; Kainski, 1960; Ostazeski, 1967). The species of *Fusarium* most frequently associated with crown and root rots of forage legumes is *F. oxysporum*, followed by *F. avenaceum*, *F. solani*, *F. acuminatum*, *F. tricinctum*, and *F. moniliforme* (Grau, 1996; Leath, 1989). In addition, *F. oxysporum* has been reported as the causal organism of Fusarium wilt of birdsfoot trefoil (Gotlieb and Dorisky, 1983; Zeiders and Hill, 1988). More recently, Bergstrom and Kalb (1995) described a wilt organism of birdsfoot trefoil as a specific pathogen of this species, for which they proposed a new taxon, *F. oxysporum* f.sp. *loti*. In Uruguay, Altier (1994, 1997) studied the fungi associated with diseased birdsfoot trefoil plants in a nursery and found that the majority of fungi isolated from crown and root tissues were *Fusarium* spp. (72%), with the two most frequently isolated species being *F. oxysporum* (54% of total) and *F. solani* (9% of total). Similar results were found by Chao *et al.* (1994), who reported *Fusarium* as the main genus (>80%) associated with infected crowns and roots during a survey of diseases affecting birdsfoot trefoil in western Uruguay and the Entre Rios Province, Argentina.

While the information published on crown and root diseases of *Lotus* focuses on descriptions of pathogens, etiology, or yield impacts, studies on disease ecology and epidemiology are limited (English, 1999). Information on the ecological aspects of Fusarium crown and root disease comes from studies done with other plant hosts. Forage legume roots are most likely colonized by *Fusarium* species shortly after planting. However, disease symptoms may not appear for some time. This delay has been generally attributed to the weak pathogenicity of root rotting *Fusaria*, which cause more severe rot when plants are under stress (Grau, 1996; Leath, 1989). Fusarium rot then progresses gradually, increasing in severity with the age of the plant (Kalb *et al.*, 1994; Leath, 1989).

Knowledge of disease development on individual plants must be coupled with epidemiological studies to understand host population responses to pathogen population pressures in the field. Although gross estimates on the impact of crown and root rots on forage legumes are available, there is much less research concerning the quantitative measurement of disease incidence and severity and the dynamics of these diseases in time and space (Nutter and Gaunt, 1996). The understanding of root disease development in time and space is of critical importance for the management of a perennial forage crop. The longevity of each plant, and therefore the productivity and persistence of the crop, will be directly dependent on healthy root systems. Until epidemiological data are available, disease management in forages will be imprecise (Leath, 1989).

Quantitative characterization of root disease epidemics has been difficult because of the relative inaccessibility of the roots (Campbell, 1986). The progress of root disease epidemics is most commonly monitored as an increase in incidence and severity of root symptoms. Obtaining these measurements requires destructive sampling which does not allow repeated assessments on the same plant. Sampling strategies must be carefully designed in order to study the temporal development and spatial pattern of disease (Campbell and Madden,

1990).

The current study was aimed to assess the importance of crown and root rot complex on birdsfoot trefoil production in Uruguay and characterize disease epidemiology. This knowledge should provide insight into potential means of disease management to aid farmers in decision-making. Specifically, the major objectives of this research were to determine (1) the incidence and severity of crown and root diseases of birdsfoot trefoil as affected by stand age in diverse ecological regions of Uruguay, and (2) the main pathogens associated with diseased plants.

Materials and Methods

Field survey

Between September 1994 and March 1996, 12 birdsfoot trefoil fields were surveyed in three areas representing distinct physiographic regions of Uruguay, distinguished by soil type, topography, and prevalent production systems. These fields where located in areas near INIA La Estanzuela, Colonia, INIA Tacuarembó, Tacuarembó, and INIA Treinta y Tres, Treinta y Tres (INIA, National Institute for Agricultural Research, Uruguay) (Figure 2).

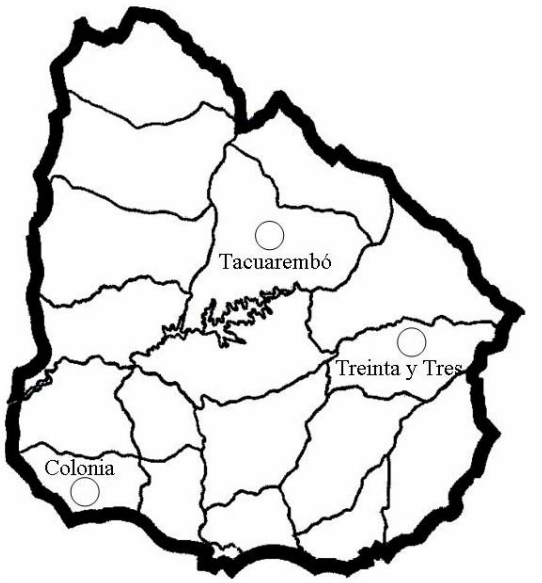

Figure 2. Location of three regions in Uruguay, in which birdsfoot trefoil was surveyed for crown and root diseases from 1994 to 1996.

Characteristics at each area are as follows; Colonia: clay-loamy, horizon B textural soils (O.M.=2.1-4.3%; pH=5.8-7.0; Al<0.1meq/100g; P>5ppm), sown pasture for very intensive production systems (livestock-crop and dairy farms); Tacuarembó: sandy/sand-loamy, deep soils (O.M.=1.1-3.3%; pH=4.5-5.5; Al=0.4-0.8meq/100g; P<5ppm), sown pasture for intensive to more extensive production systems (livestock, small area under crop rotation and dairy farms); Treinta y Tres: loamy/loam-sandy soils (O.M.=1.5-4.7%; pH=5.1-5.6; Al=0.1-0.5meq/100g; P<5ppm; high erosion risk), sown pasture for diversified production systems (livestock, hay-seed production of forage crop).

Each September (spring 1994 and 1995), birdsfoot trefoil fields were selected to compose a matrix of nine sites: three locations and three ages of stand (1-, 2-, and 3-yr-old). One-yr-old stands represented pastures sown during the fall months (April-May-June) of the current year. In September 1995 at each location, the 3-yr-old field was dropped while a new 1-yr-old field was included, in order to keep the nine site matrix. This new matrix was sampled at September 1995 and at March 1996.

A stratified sampling design was employed using twelve permanent 5x5 m quadrats per site. In each site, stand counts and plant samplings were performed twice a year, at the end of winter (September) and at the end of summer (March). Stand counts (no. of plants/m^2) were performed in the central square meter of the quadrat, using a 1.0x0.1 m transect. Sample size in each quadrat was 25 plants, with one plant randomly sampled from each 1x1 m cell of the quadrat. Plants were dug and removed with the entire root system, placed in ice chests and taken to the laboratory. At the laboratory, roots and crowns were washed and split longitudinally. Each plant was scored for disease severity (crown rot and root rot separately) following a visual five-class scale: 0 = no disease, 1 = slight rot or discoloration (less than 30% affected tissue), 2 = moderate crown/root rot or discoloration (30-70% affected tissue), 3 = severe crown/root rot or discoloration (more than 70% affected tissue), 4 = plant dead. Disease incidence (crown rot and root rot separately) was calculated as percentage of diseased plants per quadrat. The assessed unit was the whole crown or root: a scale value of 1 or higher constituted disease. Variance-to-mean ratios were calculated for the crown and root severity data for each quadrat at each sampling time to provide insight into the spatial pattern of the disease among quadrats (Campbell and Madden, 1990).

Descriptive statistics and analyses of variance (general linear model procedure, SAS Institute) were performed on crown and root rot incidence (CRI, RRI), crown and root rot severity (CRS, RRS), stand counts (NP), and variance-to-mean ratios for crown and root rot severity (VMC, VMR). Mean separations were performed using Fisher's protected LSD test ($P<0.05$). For the seven variables analyzed (CRI, RRI, CRS, RRS, NP, VMC, VMR), the variances were not homogeneous among different years (1994-1995 vs. 1995-1996) and different sampling seasons (September vs. March), therefore data were analyzed separately.

Fungal isolations

Subsamples of diseased roots from each quadrat of each site, sampled in September of 1994 and September of 1995, were used for fungal isolation. The roots were randomly selected from those representing the median severity class in the given quadrat, most commonly roots in classes 1 and 2. Pieces of 0.5-1.0 cm^2 from the interface of infected and non-symptomatic tissue were washed under flowing tap water overnight, surface-sterilized by soaking in 95% ethanol for 1 min, then soaking in 1% sodium hypochlorite for 3 min, followed by a rinse in sterile distilled water, and finally plated on PDA. Two and five pieces were plated per quadrat, for roots sampled in September of 1994 and September of 1995, respectively. The intention was to obtain at least one fungal isolate per quadrat per site (12 quadrats x 3 locations x 3 stand ages = 108 isolates). Hyphal tip growth of each different fungal colony (except for easily identified genera) was transferred to PDA plates and tubes for further

identification and storage. Each year (1994 and 1995) a collection of *Fusarium* spp. isolates was maintained on PDA slants at 4 C during the identification process (four months). Subsequently, selected isolates were stored on silica gel crystals at 5 C until needed (Windels, 1992). Identification of *F. oxysporum* was done using the procedures outlined by Nelson *et al.* (1983). Three randomly selected isolates were sent to the International Mycological Institute (IMI-CAB International, UK) for confirmation of identification (IMI No. 368015, 368016, 368017, report from Dr. D. Brayford). Two collections of *F. oxysporum* isolates were finally composed.

Results

Field survey

Crown and root rot occurred in every field surveyed, independent of location and stand age. Locations followed no consistent trend as a source of variation for crown and root rot incidence. Stand age had a large and significant effect on disease incidence (Table 1). At both sampling times (September and March), crown rot incidence and root rot incidence were significantly lower in 1-yr-old stands than in 2- or 3-yr-old stands. The largest increase in crown rot incidence was observed between September and March of 1-yr-old stands, i.e. after the first summer of the crop, when incidence reached levels close to 80% (Figure 3A). Root rot incidence increased more slowly than crown rot incidence, but by September, in two-yr-old stands, levels were very close to 100% (Figure 3A). From that time on, levels of crown and root rot incidence were always higher than 90% (Figure 3B) but differences in disease incidence between 2-yr-old vs. 3-yr-old stands were still usually significant (Table 1).

Disease severity data showed similar trends as disease incidence (Table 2, Figure 4). Location did not always have a significant or consistent effect on crown rot and root rot severity. Crown rot severity as well as root rot severity increased significantly with the age of the stand. As compared with disease incidence, disease severity continued to increase gradually from 2-yr-old stands to 3-yr-old stands, when there were no or few nondiseased plants left (disease incidence was close to 100%). The largest increase in disease severity occurred during the winter for both years. Crown rot severity was always higher than root rot severity, except in September in 1-yr-old stands, where crown rot severity was equal to or lower than root rot severity.

Stand counts were significantly affected by location and stand age (Table 3). Two and 3-yr-old stands at Tacuarembó generally had lower counts than the other two locations. The average number of plants per square meter declined as stands aged. The largest reduction was observed from 2- to 3-yr-old stands. Within each field, the stand counts indicated that most plants died during the summer, as determined by large differences between September counts and March counts (Figure 5). Data on stand counts showed the reverse trend from data on disease level, i.e. the older the stand, the higher the disease level and the lower the number of plants per square meter.

Table 1. Incidence[1] of Fusarium crown and root rot in birdsfoot trefoil, for each sampling time[2] as affected by stand age.

	Crown Rot Incidence (%)				Root Rot Incidence (%)			
Stand Age	**S942**	**M95**	**S95**	**M96**	**S94**	**M95**	**S95**	**M96**
1-yr-old	16[3]	78	2	80	17	43	26	79
2-yr-old	96	95	100	99	91	92	96	99
3-yr-old	99	99	100	100	99	98	99	100
LSD (0.05)	2	3	1	4	3	4	4	4
CV (%)	7	8	4	8	9	12	11	8

[1] Disease incidence: No. of diseased plants/total No. of assessed plants.
[2] September 1994 (S94), March 1995 (M95), September 1995 (S95), March 1996 (M96). Data were from the same 9 site matrix for Sept. 94 vs. March 95, and for Sept. 95 vs. March 96, so these two pairs of columns of values can be compared. However, variances were not homogeneous, therefore LSDs were not calculated.
[3] Average of three locations.

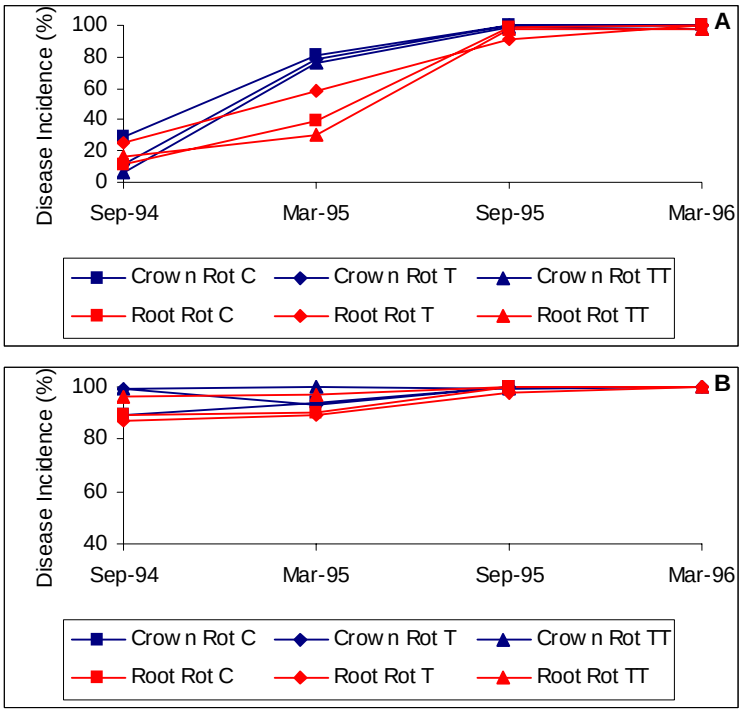

Figure 3. Progress of disease incidence of crown and root rot of birdsfoot trefoil in 1- and 2-yr-old stands **(A)** and 2- and 3-yr-old stands **(B)**, surveyed in Colonia (C), Tacuarembó (T) and Treinta y Tres (TT). Disease incidence was calculated as No.diseased plants/total No. assessed plants.

Table 2. Severity[1] of Fusarium crown and root rot in birdsfoot trefoil, for each sampling time[2] as affected by stand age.

	Crown Rot Severity				Root Rot Severity			
Stand Age	**S942**	**M95**	**S95**	**M96**	**S94**	**M95**	**S95**	**M96**
1-yr-old	0.17[3]	0.90	0.02	1.06	0.18	0.48	0.28	0.97
2-yr-old	1.33	1.49	1.82	1.91	1.20	1.26	1.43	1.65
3-yr-old	1.67	1.75	2.22	2.39	1.52	1.49	1.80	2.00
LSD (0.05)	0.06	0.07	0.08	0.14	0.07	0.09	0.09	0.13
CV (%)	12.0	11.4	11.9	16.0	15.5	16.8	16.4	17.4

[1] Disease severity: 0 = no disease, 1 = slight crown/root rot (<30% affected tissue), 2 = moderate crown/root rot (30-70% affected tissue), 3 = severe crown/root rot (>70% affected tissue), 4 = plant dead. Since the scale included a class 0, crown and root rot severity are expressed as a disease index (DSI).
[2] September 1994 (S94), March 1995 (M95), September 1995 (S95), March 1996 (M96). Data were from the same 9 site matrix for Sept. 94 vs. March 95, and for Sept. 95 vs. March 96, so these two pairs of columns of values can be compared. However, variances were not homogeneous, therefore LSDs were not calculated.
[3] Average of three locations.

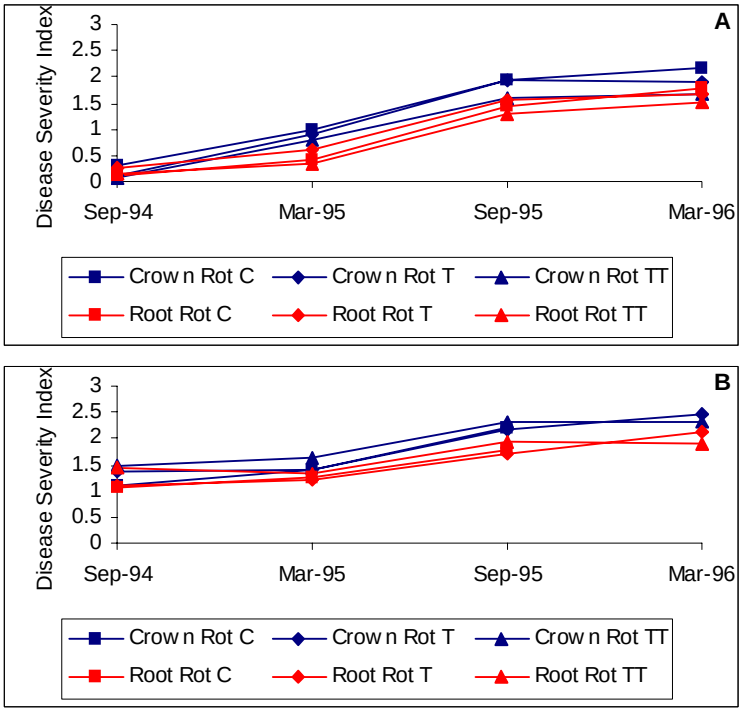

Figure 4. Progress of disease severity of crown and root rot of birdsfoot trefoil in 1-and 2-yr-old stands **(A)** and 2- and 3-yr-old stands **(B)**, surveyed in Colonia (C), Tacuarembó (T) and Treinta y Tres (TT). Disease severity was rated using a visual 5-class scale: 0 = no disease, 1 = slight crown/root rot (<30% affected tissue), 2 = moderate crown/root rot (30-70% affected tissue), 3 = severe crown/root rot (>70% affected tissue), 4 = plant dead. Since the scale included a class 0, crown and root rot severity are expressed as a disease index (DSI).

Table 3. Number of plants of birdsfoot trefoil per square meter, for each sampling time[1] as affected by stand age.

	No. of plants/m^2			
Stand Age	**S941**	**M95**	**S95**	**M96**
1-yr-old	314[2]	168	188	106
2-yr-old	214	116	146	63
3-yr-old	89	46	86	1
LSD (0.05)	25	16	21	15
CV (%)	26	31	32	47

[1] September 1994 (S94), March 1995 (M95), September 1995 (S95), March 1996 (M96). Data were from the same 9 site matrix for Sept. 94 vs. March 95, and for Sept. 95 vs. March 96, so these two pairs of columns of values can be compared. However, variances were not homogeneous, therefore LSDs were not calculated.
[2] Average of three locations.

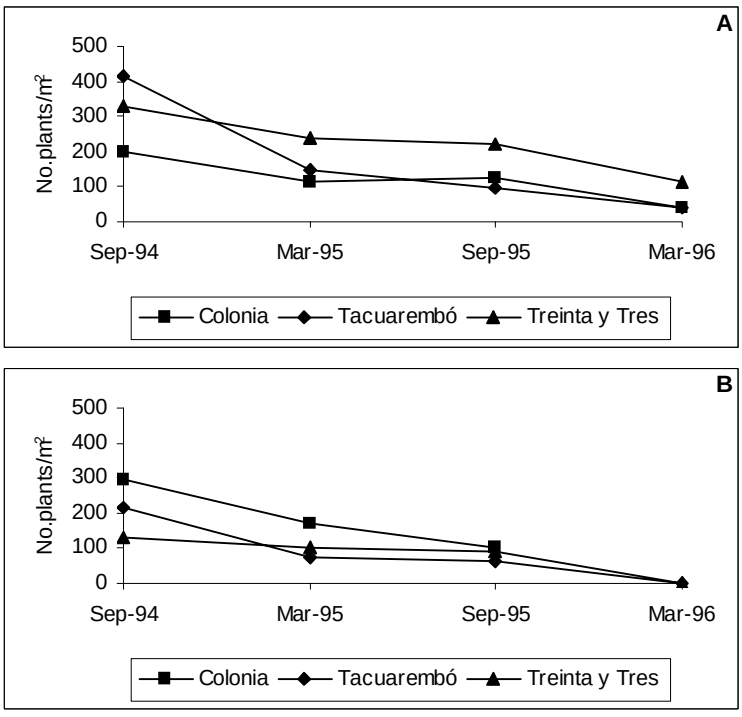

Figure 5. Reduction in the number of plants of birdsfoot trefoil in 1- and 2-yr-old **(A)** and 2- and 3-yr-old **(B)** stands surveyed in Colonia, Tacuarembó and Treinta y Tres.

The average variance-to-mean (VM) ratios were always less than 1, which suggests a rather uniform distribution of the disease independent of sampling time, stand age, and location (Table 4). Location did not have a significant effect on VM ratios, except for March 96. However, VM ratios were significantly affected by stand age, the younger the stand the higher the VM ratio. The highest average VM ratios recorded in September of 1-yr-old stands were closest to 1, which is indicative of a nearly random pattern of the disease in new stands. VM ratios for crown rot severity tended to be slightly lower than VM ratios for root rot severity, suggesting a marginally more uniform distribution of crown rot than root rot.

Table 4. Variance to mean ratios (V/M) of Fusarium crown and root rot severity in birdsfoot trefoil, for each sampling time[1] as affected by stand age.

	V/M Crown Rot Severity				V/M Root Rot Severity			
Stand Age	**S941**	**M95**	**S95**	**M96**	**S94**	**M95**	**S95**	**M96**
1-yr-old	0.89[2]	0.34	0.26	0.48	0.80	0.67	0.77	0.45
2-yr-old	0.24	0.28	0.23	0.28	0.31	0.32	0.26	0.28
3-yr-old	0.20	0.19	0.16	0.17	0.21	0.21	0.23	0.23
LSD (0.05)	0.08	0.04	NS	0.07	0.09	0.06	0.07	0.07
CV (%)	40.9	33.8	119.0	42.8	41.9	31.8	37.6	41.8

[1] September 1994 (S94), March 1995 (M95), September 1995 (S95), March 1996 (M96). Data were from the same 9 site matrix for Sept. 94 vs. March 95, and for Sept. 95 vs. March 96, so these two pairs of columns of values can be compared. However, variances were not homogeneous, therefore LSDs were not calculated.
[2] Average of three locations.

Fungal isolations

Fungal colonies were recovered from root and crown pieces of plants sampled in all three locations. Independent of the location, root and crown pieces from 1-yr-old plants yielded few fungal colonies (11.9% and 14.9% of the total, for 1994 and 1995, respectively), while fungi were readily isolated from root and crown pieces from 2- and 3-yr-old plants.

The majority of fungi isolated from diseased crown and root tissues of birdsfoot trefoil were *Fusarium* spp., with the most frequently and consistently isolated species being *F. oxysporum* (Table 5). The second most frequently isolated fungi included presumed saprophytic genera, *Penicillium*, *Aspergillus*, *Gliocladium*, *Epicoccum*, *Cladosporium*, *Rhizopus*, and *Mucor*. Unknown fungi included sterile hyphomycetes and coenocytic, nonsporulating species, and were recovered in relatively low frequencies (Table 5). One isolate recovered in September 1994, identified tentatively as *Mycoleptodiscus* spp., and two isolates recovered in September 1994, identified presumptively as *Rhizoctonia solani*, were counted as unknown fungi.

Table 5. Percent frequency of fungi isolated from diseased crowns and roots of birdsfoot trefoil plants from three locations (Colonia, Tacuarembó and Treinta y Tres) in Uruguay, for two sampling dates (September 1994 and September 1995).

	Percent frequency	
Fungi isolated	**1994**	**1995**
Fusarium	53	57
F. oxysporum	35	40
Other *Fusarium* spp.	18	17
Presumed saprophytic genera[1]	35	38
Unknown[2]	12	5
Total number of isolates[3]	120	365
Number of pieces examined	216	540

[1] Species of *Penicillium*, *Aspergillus*, *Epicoccum*, *Cladosporium*, *Rhizopus*, *Mucor*, *Gliocladium*, and others.
[2] Sterile hyphomycetes, coenocytic, nonsporulating fungi and others.
[3] Total number of yielding colonies on PDA from 0.5-1.0 cm^2 pieces cut from surface-sterilized diseased birdsfoot trefoil crowns and roots.

Sixty four *Fusarium* spp. isolates were recovered from roots sampled in 1994, and 208 isolates from roots sampled in 1995, and composed the two *Fusarium* spp. collections. Forty two *F. oxysporum* isolates from 1994, and 146 isolates from 1995 composed the two *F. oxysporum* collections.

Discussion

Deterioration of roots and crowns of birdsfoot trefoil was demonstrated to occur in diverse ecological areas of the country, representing distinct physiographic regions distinguished by soil type, topography, and prevalent production systems. The range of edaphic conditions surveyed does not limit the development of crown and root diseases in birdsfoot trefoil. We confirm the hypothesis that the occurrence of the crown and root rot complex is a widespread phenomenon in Uruguay and has a negative impact on birdsfoot trefoil production and persistence (Altier, 1994; 1997). Our results also agree with preliminary information obtained by Chao *et al.* (1994) during a survey of diseases affecting 12 birdsfoot trefoil pastures in western Uruguay and the Entre Rios Province, Argentina, which reported that crown and root rot were the most prevalent diseases.

The repeated isolation of *Fusarium oxysporum* from symptomatic plants of birdsfoot trefoil suggests this species is frequently responsible for crown and root rot and stand decline. No other known pathogen that is alone capable of causing these disease symptoms was isolated

from diseased crown and root tissues. These results are consistent with previous findings when studying the fungi associated with crown and root rot complex of birdsfoot trefoil and other forage legumes (Altier, 1994; 1997; Beuselinck, 1988; Chao *et al.*, 1994; Grau, 1996; Kainski, 1960; Leath, 1989; Zeiders and Hill, 1988).

The random initial spatial pattern of crown and root rot (variance to mean ratios slightly less than 1) may result from the ubiquitous nature of *F. oxysporum* and indicates that the potential for disease development is independent of location and site. The fact that VM ratios decreased with the age of the stand suggests a gradual saturation of the system, where every plant sampled was diseased as a consequence of the dispersal over space and time. The observed lower VM ratios for crown rot severity as compared with VM ratios for root rot severity indicates that infection of crown tissue progresses faster than infection of root tissue and saturation of the system occurs early.

The large and significant effect of stand age on disease incidence and severity was expected and previously reported (Berkenkamp *et al.*, 1972; Beuselinck, 1988; Drake, 1958; Grant and Marten, 1985; Grau, 1996; Henson, 1962; Hill and Zeiders, 1987; Hoveland *et al.*, 1982; Hoveland *et al.*, 1987; Kainski, 1960; Leath, 1989; Leath *et al.*, 1971; Miller *et al.*, 1964; Pettit *et al.*, 1966; Taylor *et al.*, 1973). However, we did not expect the high disease incidence levels as early as March, when the plants had not completed one year in the field. Root rot incidence increased slower than crown rot incidence, but by September in the second production year most of the plants were symptomatic. Large areas of necrosis limit the amount of healthy tissue available to maintain the essential physiological functions of water and nutrient absorption, nitrogen fixation, carbohydrate storage and translocation to the growing points (Grau, 1996).

In the conditions of Uruguay, summer appears to be the critical season for plant survival. While the largest increases in crown and root rot severity occurred during the winter, stand count results indicate that most plants died during the summer. The high soil temperatures registered during that season, interacting with periods of drought, most likely accentuate the stress on plants already weakened by disease. Since data on stand counts supported data on disease level, i.e. the older the stand, the higher the disease level and the lower the number of plants per square meter, a relationship between disease level and persistence in the field is clearly established.

Fusarium crown and root rot is affected by environmental, biological and management factors that stress the plant and is a chronic rather than an acute disease (Grau, 1996; Leath, 1989). The utilization of perennial forage legumes is in itself the most serious stress repetitively imposed on the plants (Beuselinck *et al.*, 1984; Hoveland, 1989; Leath, 1989; Miller *et al.*, 1964; Taylor *et al.*, 1973). In the production systems of Uruguay, pastures are utilized during most of the year, therefore grazing animals are imposing a severe stress on the legume. Animals influence legume performance by selective grazing, trampling and excretion (Hoveland, 1989). Animal trampling causes direct injuries to the crown of the plants producing entry points for pathogens. Despite the fact that *Fusarium* species may directly penetrate unwounded tissues, wounding alters the host-pathogen interaction and favors fungal development in the tissues (Chi *et al.*, 1964; Stutz *et al.*, 1985). Wounding of

roots and crowns is a common phenomenon and *F. oxysporum* is a wound parasite that can readily invade tissues (Kalb *et al.*, 1994; Leath, 1989; Leath and Hower, 1993). *Fusarium* spp. are primarily cortical invaders which can survive and increase in the cortex until conditions favor pathogenicity (Kommedahl and Windels, 1979). Once the infection has taken place, the plant remains diseased. The impact of the disease and the rate at which it develops are functions of the environment and management. Because climate cannot be impacted, proper management becomes the prime strategy. Correct and timely application of crop management practices during the winter and summer months must contribute to reduce the stresses imposed on the plants and therefore, to reduce the rate of disease development and stand decline. Crop management practices, such as frequency and intensity of utilization, play a role in the development of Fusarium crown and root rot of red clover (Fezer, 1961; Fulton and Hanson, 1960; Rufelt, 1986; Siddiqui *et al.*, 1968) and alfalfa (Lukezic *et al.*, 1969). The effect of utilization management on the rate of disease development and consequently the impact on birdsfoot trefoil productivity need to be investigated.

Additionally, phenotypic selection has proved to be effective in increasing the level of resistance to *F. oxysporum*, when developing birdsfoot trefoil populations (Altier *et al.*, 2000; Rebuffo and Altier, 1997; Zeiders and Hill, 1988). The release of cultivars with enhanced resistance need to be coupled with improved management practices to provide an integrated management scheme for Fusarium crown and root diseases.

References

ALTIER N. 1994. Current status of research on *Lotus* diseases in Uruguay. **In** BEUSELINCK P.R. and ROBERTS C.A. (Eds.) The First International *Lotus* Symposium, Proceedings. University of Missouri, St. Louis, USA. pp. 203-205.

ALTIER N. 1997. Enfermedades del lotus en Uruguay. [Diseases of lotus in Uruguay] INIA, Montevideo, Uruguay. *Serie Técnica*, **93**. 16 p. [In Spanish]

ALTIER N.A., EHLKE N.J. and REBUFFO M. 2000. Divergent selection for resistance to Fusarium root rot in birdsfoot trefoil. *Crop Science*, **40**, 670-675.

BERGSTROM G.C. and KALB D.W. 1995. *Fusarium oxysporum* f.sp. *loti*: a specific wilt pathogen of birdsfoot trefoil in New York. *Phytopathology*, **85**, 1555.

BERGSTROM G.C., KALB D.W., TILLAPAUGH B.P. and LUCEY R.F. 1995. Fusarium wilt of birdsfoot trefoil: biology and prospects for management. **In** Proceedings of the 11th Eastern Forage Improvement Conference. (Ottawa, Ontario, Canada). pp. 43-44.

BERKENKAMP B., FOLKINS L. and MEERES J. 1972. Crown and root rot of birdsfoot trefoil in Alberta. *Canadian Plant Disease Survey*, **52**, 1-3.

BEUSELINCK P.R. 1988. Fungi associated with birdsfoot trefoil. *Lotus Newsletter*, **19**, 11-14.

BEUSELINCK P.R., PETERS E.J. and MCGRAW R.L. 1984. Cultivar and management effects on stand persistence of birdsfoot trefoil. *Agronomy Journal*, **76**, 490-492.

CAMPBELL C.L. 1986. Interpretation and uses of disease progress curves for root diseases. **In** LEONARD K.J. and FRY W.E. (Eds.) Plant disease epidemiology. Vol 1: Population dynamics and management. New York, Macmillan. pp. 38-54.

CAMPBELl C.L. and MADDEN L.V. 1990. Plant disease epidemiology. New York, John Wiley & Sons. 532 p.

CHAO L., DE BATTISTA J.P. and SANTIÑAQUE F. 1994. Incidence of birdsfoot trefoil crown and root rot in west Uruguay and Entre Rios (Argentina). **In** BEUSELINCK P.R. and ROBERTS C.A. (Eds.) The First International *Lotus* Symposium, Proceedings. University of Missouri, St. Louis, USA. pp. 206-209.

CHI C.C., CHILDERS W.R. and HANSON E.W. 1964. Penetration and subsequent development of three *Fusarium* species in alfalfa and red clover. *Phytopathology*, **54**, 434-437.

DRAKE C.R. 1958. Diseases of birdsfoot trefoil in six southeastern states in 1956 and 1957. *Plant Disease Reporter*, **42**, 145-146.

DRAKE C.R. 1961. Rhizoctonia crown rot of birdsfoot trefoil (*Lotus corniculatus*). *Plant Disease Reporter*, **45**, 572-573.

ENGLISH J.T. 1999. Diseases of *Lotus*. **In** BEUSELINCK P.R. (Ed.) Trefoil: the science and technology of *Lotus*. CSSA Special Publication 28. Madison, WI., ASA-CSSA. pp. 121-131.

FEZER K.D. 1961. Common root rot of red clover: pathogenicity of associated fungi and environmental factors affecting susceptibility. Cornell University Agricultural Experiment Station, NY, USA. *Memoir* **377**.

FORMOSO F. 1993. *Lotus corniculatus*. I. Performance forrajera y características agronómicas asociadas. [Forage performance and associated agronomic traits] INIA, Montevideo, Uruguay. *Serie Técnica*, **37**. 20 p. [In Spanish]

FULTON N.D. and HANSON E.W. 1960. Studies on root rots of red clover in Wisconsin. *Phytopathology*, **50**, 541-550.

GOTLIEB A.and DORISKY H. 1983. Fusarium wilt of birdsfoot trefoil in Vermont and New York. *Phytopathology*, **73**, 366.

GRANT W.F. and MARTEN G.C. 1985. Birdsfoot trefoil. **In** HEATH M.E., BARNES R.F. and METCALFE D.S. (Eds.). Forages: the science of grassland agriculture. 4th. ed. Ames, Iowa State University Press. pp. 98-108.

GRAU C.R. 1996. Disease complexes. **In** CHAKRABORTY S., LEATH K.T., SKIPP R.A., PEDERSON G.A., BRAY R.A., LATCH G.C.M. and NUTTER F.W.Jr. (Eds). Pasture and forage crop pathology. ASA-CSSA-SSSA: Madison, WI, USA. pp. 453-472.

HENSON P.R. 1962. Breeding for resistance to crown and root rots in birdsfoot trefoil, *Lotus corniculatus* L. *Crop Science*, **2**, 429-432.

HILL R.R.Jr. and ZEIDERS K.E. 1987. Among- and within-population variability for forage yield and *Fusarium* resistance in birdsfoot trefoil. *Genome*, **29**, 761-764.

HOVELAND C.S. 1989. Legume persistence under grazing in stressful environments of the United States. **In** MARTEN G.C., MATCHES A.G., BARNES R.F., BROUGHAM R.W., CLEMENTS R.J. and SHEATH G.W. (Eds.) Persistence of forage legumes. Madison, ASA-CSSA-SSSA, Madison, WI, USA. pp. 375-383.

HOVELAND C.S., ALISON M.W.Jr. and DOBSON J.W.Jr. 1987. Performance of birdsfoot trefoil varieties in North Georgia. Athens, Agricultural Experiment Stations, University of Georgia. *Research Report*, **528**. 5 p.

HOVELAND C.S., HAALAND R.L., HARRIS R.R. and MCGUIRE J.A. 1982. Birdsfoot trefoil in Alabama. Auburn, Alabama Agricultural Experiment Station, Auburn University. *Bulletin* **537**.

KAINSKI J.M. 1960. Study of fungi involved in root rots and seedling diseases of birdsfoot trefoil. Cornell University Agricultural Experiment Station, NY, USA. *Memoir*, **369**.

KALB D.W., BERGSTROM G.C. and SHIELDS E.J. 1994. Prevalence, severity, and association of fungal crown and root rots with injury by clover root curculio in New York alfalfa. *Plant Disease*, **78**, 491-495.

KOMMEDAHL T. and WINDELS C.E. 1979. Fungi: pathogen or host dominance in disease. **In** KRUPA S.V. and DOMMERGUES Y.R. (Eds.). Ecology of root pathogens. Elsevier Scientific Publishing Company: Amsterdam, The Neatherlands. pp. 1-103.

LEATH K.T. 1989. Diseases and forage stand persistence in the United States. **In** MARTEN G.C., MATCHES A.G., BARNES R.F., BROUGHAM R.W., CLEMENTS R.J. and SHEATH G.W. (Eds.) Persistence of forage legumes. ASA-CSSA-SSSA: Madison, WI, USA. pp. 465-479.

LEATH K.T.and HOWER A.A. 1993. Interaction of *Fusarium oxysporum* f.sp. *medicaginis* with feeding activity of clover root curculio larvae in alfalfa. *Plant Disease*, **77**, 799-802.

LEATH K.T., LUKEZIC F.L., CRITTENDEN H.W., ELLIOT E.S., HALISKY P.M., HOWARD F.L. and OSTAZESKY S.A. 1971. The Fusarium root rot complex of selected forage legumes in the *Northeast.* Pennsylvania State University, PA, USA. *Bulletin* **777**.

LEATH K.T., WELTY R.E., PRATT R.G. and SONODA R.M. 1996. Pasture/forage crops and diseases in the United States. **In** CHAKRABORTY S., LEATH K.T., SKIPP R.A., PEDERSON G.A., BRAY R.A., LATCH G.C.M. and NUTTER F.W.Jr. (Eds.) Pasture and forage crop pathology. ASA-CSSA-SSSA: Madison, WI, USA. pp. 33-58.

LUKEZIC F.L., BLOOM J.R. and CARROLL R.B. 1969. Development of *Fusarium* root rot and its effect on root and crown carbohydrate levels of clipped alfalfa plants grown in a gnotobiotic environment. *Phytopathology*, **59**, 1575-1579.

MILLER J.D., KREITLOW K.W., DRAKE C.R. and HENSON P.R. 1964. Stand longevity studies with birdsfoot trefoil. *Agronomy Journal*, **56**, 137-139.

NELSON P.E., TOUSSOUN T.A. and MARASAS W.F.O. 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press: University Park, PA, USA.

NUTTER F.W.Jr. and GAUNT R.E. 1996. Recent developments in methods for assessing disease losses in forage/pasture crops. **In** CHAKRABORTY S., LEATH K.T., SKIPP R.A., PEDERSON G.A., BRAY R.A., LATCH G.C.M. and NUTTER F.W.Jr. (Eds.) Pasture and forage crop pathology. ASA-CSSA-SSSA, Madison, WI, USA. pp. 93-118.

OSTAZESKI S.A. 1967. An undescribed fungus associated with a root and crown rot of birdsfoot trefoil (*Lotus corniculatus*). *Mycologia*, **59**, 970-975.

PETTIT R.E., CALVERT O.H. and BALRIDGE J.D. 1966. *Leptodiscus terrestris* colonization of birdsfoot trefoil roots in Missouri. *Plant Disease Reporter*, **50**, 753-755.

REBUFFO M. and ALTIER N. 1997. Breeding for persistence in *Lotus corniculatus*. **In** Proceedings of the XVIII International Grassland Congress. Winnipeg and Saskatoon, Canada. pp. 4/73-74.

RUFELT S. 1986. A threshold value for cutting intensity in relation to *Fusarium* root rot in red clover. **In** Studies on *Fusarium* root rot of red clover (*Trifolium pratense* L.) and the potential for its control. Ph.D. Thesis. Uppsala, Swedish University of Agricultural Sciences. *Plant Protection Reports, Dissertation*, **10**.

SAS/STAT®. 1997. Software: changes and enhancements through Release 6.12. (SAS Institute: Cary, NC, USA).

SIDDIQUI W.M., HALISKY P.M. and LUND S. 1968. Relationship of clipping frequency to root and crown deterioration in red clover. *Phytopathology*, **58**, 486-488.

STUTZ J.C., LEATH K.T. and KENDALL W.A. 1985. Wound-related modifications of penetration, development, and root rot by *Fusarium roseum* in forage legumes. *Phytopathology*, **75**, 920-924.

TAYLOR T.H., TEMPLETON W.C.Jr. and WYLES J.W. 1973. Management effects on persistence and productivity of birdsfoot trefoil (*Lotus corniculatus* L.). *Agronomy Journal*, **65**, 646-648.

VIANDS D.R., EHLKE N.J., PAPADOPOULOS Y.A. and SMITH R.R. 1994. Cooperative project to develop birdsfoot trefoil with multiple disease resistance. *Lotus Newsletter*, **25**, 45-46.

WINDELS C.E. 1992. *Fusarium*. **In** SINGLETON L.L., MIHAIL J.D. and RUSH C.M. (Eds). Methods for research on soilborne phytopathogenic fungi. APS Press: St. Paul, MN, USA. pp. 115-128.

ZEIDERS K.E. and HILL R.R.Jr. 1988. Measurement of resistance to *Fusarium* wilt/root and crown rot in birdsfoot trefoil populations. *Crop Science*, **28**, 468-473.