# BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS*) COVER FOR ALABAMA CROPPING SYSTEMS: FUNGAL DISEASES, SUSCEPTIBILITY TO NEMATODES, AND EFFICACY OF HERBICIDES

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## Abstract

*Lotus corniculatus* (birdsfoot trefoil) is a common flowering plant in the pea family Fabaceae and native to Eurasia and North Africa. It is used in agriculture as a forage plant and also grown for pasture, hay, and silage due to its non-bloating properties; along with being used as a cover crop. Auburn University's breeding program for birdsfoot trefoil is attempting to extend the forage's geographic adaptation across the southern United States. Stand decline of the birdsfoot trefoil breeding lines due to fungal diseases and nematode pressure was observed at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee, Alabama in the 2015 season. We were able to isolate *Macrophomina phaseolina* from symptomatic plants. This pathogen causes stand decline, root rot, and charcoal rot in more than 500 crop and non-crop host plants (Smith and Carvil, 1977). The successful completion of Koch's Postulates indicates *Fusarium oxysporum* is a causal agent of seedling disease resulting in stand decline of birdsfoot trefoil in Alabama. *Meloidogyne incognita* (root-knot nematode) had a higher population and reproductive factor and can increase *M. incognita* populations on three cultivars of birdsfoot trefoil tested. Two herbicides (Butyrac 200 and Panoramic) reduced the fresh shoot mass of cultivars tested in our greenhouse trial along with the Pardee cultivar being the most susceptible to herbicide damage.

## Introduction

### Background on Birdsfoot trefoil

Birdsfoot trefoil (BFT) has been used as a cool-season forage in North America for a considerable amount of time and shares a distributional range with alfalfa (*Medicago sativa*). BFT grows new shoots from axillary buds located on the stems rather than from crown or basal buds as in alfalfa (Nelson and Smith, 1968b). Carbohydrate reserves needed for regrowth during the growing season are minimal within the root of BFT compared to other legumes (Nelson and Smith, 1968a; Smith, 1962). However, BFT is adapted to circumstances where defoliation is regular, such as grazed pastures, because it maintains a higher amount of residual leaf area near the soil surface than alfalfa. Forage managers since the early 1960s have used the saying "frequent but not close" for BFT and "close but not frequent" for alfalfa to differentiate among suitable defoliation methods and responses for these two species. The BFT normally used in forage production is a tetraploid with 2n=4x=24 chromosomes. The generally tetrasomic inheritance suggests that it is an autotetraploid but certain traits such as tannin content seem to be inherited in a disomic manner (Dalrymple et al., 1984; Fjellstrom et al., 2003). Normally bivalent pairing has been observed during meiosis (Seaney and Hanson, 1970). Favored pairing based on paternal and maternal source of the chromosomes is one explanation (Stift et al., 2008). Recent research proposes that the early assessment of qualitative inheritance may have been due to small sample size and a quantitative model could be more fitting (Miller and Ehlke, 1997). A thought is that most BFT accessions are highly allogamous, where cross-fertilization is enforced through a functioning self-incompatibility system.

### Drought/Heat Tolerance – Persistence (Trans-Regional Aspect)

For the benefit of beef production, the "summer slump" (or period of slow mid-summer growth) of cool-season grass pastures can be offset with a more efficiently deep rooted (and thus, drought-resistant), high-quality, bloat-free legume than with a high productive but lower quality C4 grass. Farmers that have experience with BFT have found it difficult to maintain productive stands which provides a major component for this project. A perennial growth habit is a stated goal for forage legume improvement, but the more significant goal should be stand longevity. A forage legume plant is not perennial in the sense of an oak tree (*Quercus* spp.), which once it has survived the juvenile phase, may live for many decades. Stand longevity is a function of the plant population. Forage legume crop management purpose is to

maintain a minimum number of plants, or shoots as in the case of alfalfa (Undersander, 2011), for a productive stand over the desired stand life. Small increases in the persistence of individual members of those populations have an impact on the longevity of a stand, as does the recruitment of seedlings. There is no evidence for autotoxicity in BFT, as there is for alfalfa, but tall fescue has been found to be allelopathic toward BFT late in the growing season (Stephenson and Posler, 1998).

**Tannin Content**

The specific tannin found in BFT increases the efficiency of plant protein utilization for ruminants (Waghorn, 2008) and improves weight gain (Douglas et al., 1995) along with preventing bloat (Fay et al., 1980), and reduces urinary N losses (Misselbrook et al., 2005; Crush and Keogh, 1998). Toxicity of alkaloids from endophyte-infested tall fescue continues to be a cattle health concern and an interference to beef production in the ETZ. A moderate to high tannin BFT could increase cattle productivity, especially in endophyte-infested tall fescue pastures, where it has been demonstrated that more endophyte-infested tall fescue was consumed by ruminants when BFT was present than when no BFT was available (Owens et al., 2012; Wen et al., 2002).

**Fungal Diseases**

Diseases of *Lotus* are caused by pathogens dispersed among at least 19 fungal genera and they occur wherever *Lotus* is managed. All vegetative and reproductive parts of *Lotus* plants are vulnerable to at least one of these pathogens. For example, *Colletotrichum*, *Cercospora*, *Stemphylium*, and *Pseudopeziza* infect foliage of *Lotus* and other forage legumes. It is possible to find two or more diseases caused by these pathogens occurring simultaneously but the limited literature does not specify how often they occur alone or as complexes on *Lotus*. The probability of two or more pathogens co-infecting *Lotus* depends significantly on the microclimate of the forage canopy. For example, in Missouri during periods of high humidity and dew formation, it is common to find both foliar blight and leaf spot caused by *Rhizoctonia solani* and *S. loti*, respectively.

The impacts of fungal plant pathogens on foliage, stem, and blossom growth and development are not well defined with few exceptions. Of the few reports in the literature, English (1992) stated that 90% or more BFT leaves produced per shoot each year could be damaged by foliar blight and leaf spot. Stewart et al. (1994) stated that yield losses could be up to 36% for BFT infected with *Colletorichum acutatum*.

Less examination of the physiological characteristics of interactions between *Lotus* and foliar fungal pathogens has occurred. The most in-depth studies in this area to date have been those of Millar and Higgins (1970). These authors described the relationships of β-glucosidase production and cyanogenesis in *Lotus* infected with *S. loti*. In contrast to foliar diseases, fungal pathogens that infect plant crowns and roots, including *Fusarium* sp., *R. solani*, *Mycoleptodiscus terrestris*, and *Macrophomina phaseolina*, occur usually in the same tissues of individual plants (Altier, 1994; Chao et al., 1994; Drake, 1958). It is unusual to find a single pathogen associated with roots of *Lotus*. It has been difficult to identify the role of any single organism to crown and root rot because the pathogens have specific environmental and host requirements for infections. Pettit et al. (1969) gave one of the only quantified studies of the impacts of pathogen of this crown rot complex. They described during controlled experiments those variable reductions in root biomass of BFT when inoculated with individual or combined pathogens including *M. terrestris*, *R. solani*, *F. oxysporum*, and *M. phaseolina*.

*Fusarium oxysporum*, a fungal pathogen, causes a vascular wilt of *Lotus*. This disease has been described to severely reduce production of BFT in the northeastern U.S. (Murphy et al., 1985). Recent research efforts have focused on selection of resistant germplasm for this disease (Zeiders and Hill, 1988).

**Nematodes**

Nematodes that are pathogens of *Lotus* have received less attention than fungi. A larger part of the literature on nematodes has gone past survey and description and has focused on disease impacts and management. Although numerous nematode species have been described that are associated with the roots of *Lotus*, most reports have focused on two species, *Pratylenchus penetrans*, the root lesion nematode, and *Meloidogyne hapla*, the northern root knot nematode. Both of these nematodes adversely affect *Lotus* root growth and dry matter accumulation (Thompson and Willis, 1970; Townshend and Potter, 1978; Willis and Thompson, 1972). The soil environment influences the biology of these species. For example, Willis and Thompson (1969) stated that reproduction of *P. penetrans* on roots of BFT varied significantly in connection to soil moisture. These authors also found that the effects of nematode establishment on root growth and plant yield were significantly affected not only by soil moisture, but also by harvesting method.

Townshend and Potter (1978) stated that *M. hapla* reduced stand yields by causing mortality at seedling establishment and at later stages of seedling recruitment. *M. hapla* reduced yields of BFT by reducing dry matter accumulation in surviving plants. The impacts of nematode infection on plant yield and stand persistence are confounded more by interactions with root-infecting fungal pathogens such as *Fusarium* sp. (Willis and Thompson, 1975). Interactions of this type are likely to occur with other pathogens.

## Materials and Methods

### Fungi

Symptomatic stems and roots were taken from half-sib individuals located in Auburn University's field breeding plots at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee, AL and Sand Mountain Research and Extension Center in Crossville, AL. Fifteen different fungal species were identified. Thirty-eight samples were sent to be further identified and confirmed. Sixteen different species identified by DNA analysis. Five pathogenic species (*Curvalaria lunata, Fusarium oxysporum*, *Macrophomina phaseolina, Phoma* sp., *Rhizoctonia solani*) were tested in our greenhouse by Koch's postulates. The layout was a randomized complete block design with three replicates per BFT cultivar (Empire, Norcen, and Pardee). Results were analyzed using SAS 9.4 PROC GLIMMIX with means compared using Tukey-Kramer's method at $P \leq 0.05$.

### Nematodes

The pathogenicity of *Meloidogyne incognita* (root-knot), *Heterodera glycines* (soybean cyst), and *Rotylenchulus reniformis* (reniform) on birdsfoot trefoil was conducted in greenhouse trials. The experiment was a randomized complete block design with five replicates and analyzed using SAS .4 PROC GLIMMIX with means compared using Tukey-Kramer's method at $P \leq 0.05$. Inoculation amounts are as follows: *M. incognita* with 100,000 eggs/500 $cm^3$, *H. glycines* with 72,000 eggs/500 $cm^3$, and *R. reniformis* with 56,000 eggs/500 $cm^3$. Nematodes were inoculated 30 days after planting and extracted 30 days after inoculation.

### Herbicide tolerance

A greenhouse trial screening seven herbicides on three cultivars (Empire, Norcen, and Pardee) was conducted with four replications in a randomized complete block design. The following herbicides were tested at their recommended rates: Assure II at 12 oz/A, Butyrac 200 at 64 oz/A, Panoramic at 4 oz/A, Poast at 24 oz/A, Pursuit at 4 oz/A, Roundup at 8 oz/A, and Select Max at 16 oz/A. Fresh and dry total, shoot, and root biomass were taken 8 weeks after herbicide application. Results were analyzed using SAS 9.4 PROC GLIMMIX with means compared using Tukey-Kramer's method at $P \leq 0.05$.

## Results and Discussion

### *Macrophomina phaseolina*

Disease symptoms and plant ratings (1=healthy to 5=dead) were recorded weekly. Higher disease symptom ratings were observed with a significant ($P < 0.05$) increase of seedling root and hypocotyl necrosis compared between infected plants and controls (average disease rating of 2.75 for inoculated plants versus 0.83 for non-inoculated controls). All plant cultivars were equally susceptible to disease with an average 2.5, 3.0, and 2.8, respectively, at 29 days after planting.

### *Fusarium oxysporum*

Disease symptoms were observed weekly with plant disease ratings (1=healthy to 5=dead) recorded. Higher disease ratings indicated a significant ($P < 0.05$) increase of seedling decline (2.83 for plants in infested pots verses 0.83 for non-infested controls) compared between infected plants and controls. Plant survival data exhibited a significant ($P < 0.05$) difference (53% infected individuals versus 68% non-infected control plants) at 29 days after planting.

### Nematodes

Results from the greenhouse trials indicated *M. incognita* reproduced and increased population density on BFT while *R. reniformis* and *H. glycines* did not. The number of *M. incognita* eggs ranged from 116,802 to 148,505 per 500 $cm^3$ of soil on the three cultivars while J2 ranged from 355 to 927 on the three cultivars. Visual differences were observed among cultivars in some of the pots with size differences in gall development between the three cultivars of BFT. *Meloidogyne incognita* population levels ranged from 18,569 (Empire) to 40,110 (Pardee) eggs per gram of dry root. The Pardee cultivar supported less ($P < 0.05$) fresh and dry biomass (shoot/root) compared to the Norcen cultivar;

ranging from 25.10 to 51.12 g/500 $cm^3$ and 3.56 to 7.47 g/500 $cm^3$ respectively. *Meloidogyne incognita* had a significantly larger population and reproductive factor (defined as the final nematode population density divided by the initial inoculum density) than *R. reniformis* and *H. glycines*. The Empire cultivar supported the lowest populations of *M. incognita*.

**Herbicide tolerance**

Results from the greenhouse trials indicated Panoramic and Butyrac reduced the biomass of the BFT cultivars (Empire, Norcen, and Pardee). Also the results showed that the cultivar Pardee was reduced more than Empire and Norcen.

## Summary

Koch's Postulates confirmed that *M. phaseolina* is the causal agent of birdsfoot trefoil stand decline and root necrosis in Alabama. *M. phaseolina*, a species in the *Botryosphaeriaceae*, has been previously identified to infect birdsfoot trefoil in Maryland (Drake, 1958). This pathogen causes stand decline, root rot, and charcoal rot in more than 500 crop and non-crop host plants (Smith and Carvil, 1977). It is an important and wide spread pathogen of in Alabama that must be considered in birdsfoot trefoil productions systems. The successful completion of Koch's Postulates indicates *F. oxysporum* is a causal agent of seedling disease resulting in stand decline of birdsfoot trefoil in Alabama. *Fusarium* sp. have been previously identified to infect birdsfoot trefoil in Maryland, North Carolina, and Virginia (Drake, 1958) and also in New York (Roberts et al., 1956). *Meloidogyne incognita* had a high population and reproductive factor and can increase *M. incognita* populations on the three cultivars of BFT tested. This indicates that BFT is a good host for *M. incognita*. Pardee was the most limited cultivar in its growth due to *M. incognita* pressure. Tannin content could be a factor on how well nematodes infect BFT. Empire and Norcen cultivars could be used as a potential cover crop in *R. reniformis* or *H. glycines* infested fields instead of crimson clover, which allows the reproduction of these nematodes. Butyrac and Panoramic reduced the total biomass of cultivars tested but Select Max, Poast, Glyphosate, Assure II, and Pursuit did not reduce biomass.

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