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**Fungicide control of blind seed disease (*Gloeotinia temulenta*)  
without affecting AR37 endophyte in ryegrass seed crops**

**A thesis presented in partial fulfilment of the requirements for the degree of  
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in  
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## Abstract

Blind seed disease (BS) is caused by the fungus *Gloeotinia temulenta* that directly affects the germination of grass seeds by killing the embryo. This disease continues to periodically affect the forage grass seed industry (Alderman, 2001). *Epichloë* fungal infection has a symbiotic association with grasses, providing beneficial traits to the plant host, having a crucial role in ensuring the persistence of grasses against biotic and abiotic threats (Mortimer and Di Menna, 1982; Popay and Rowan, 1994). This study focuses on new fungicide testing used to control BS and its effects on the transmission of the AR37 endophyte into the new seed generation. In this study, thousand seed weights, germination percentages, blind seed determinations and immunoblot detection of endophyte were carried out to assess the effects of different foliar fungicide treatments used to control blind seed (BS) and other pathogens, on the transmission of the AR37 endophyte into the developing seed of perennial and hybrid ryegrass cultivars (Samson, Horizon and PGone50). Trial one, but not trial two, was conducted on a paddock where there were abundant buried seed with BS disease to ensure a high potential for this disease to develop in the treatments plots. In trial one, germination in Samson with all fungicide treatments used was higher, and conversely BS was lower, than the control (except T12 composed of folpet). The treatments that best controlled BS in Samson were T2 (70% germination, composed by 100 g/ha prothioconazole applied at mid-flowering); T4 (72% germination, composed by 100 g/ha prothioconazole + 250 g/ha carbendazim applied at mid-flowering and mid-seed fill); T8 (73% germination, composed by 125 g/ha azoxystrobin with 189.2 g/ha tebuconazole applied twice (at mid-flowering and mid-seed fill and 250 g/ha carbendazim at mid-seed fill); and T9 (73% germination, composed by 100 g/ha prothioconazole + 75 g/ha isopyrazam + 250 g/ha carbendazim applied at mid-flowering and mid-seed fill). No reduction in endophyte transmission to seed was observed with the fungicide treatments with the exception of the applications of folpet. In turn, with Horizon several fungicide combinations were able to improve the germination performance by controlling BS, however Horizon had a lower performance in terms of controlling BS. The percentage of Horizon seed with endophyte in all treatments was very low, possible reflecting the use of seed with a low percentage of viable AR37 endophyte when the grass seed crop was established some years previously. In trial two, germination, endophyte content, and seed yield between the treatments were not different. All treatments (including the

control) had a germination level between 84 to 89%. All treatments used in this trial maintained the AR37 endophyte content in the resultant seed lots. It is known that the application of some fungicides used to control a range of pathogens is detrimental to the viability of endophytes. Therefore, it is imperative that research in the quest of new treatments that control effectively BS without exerting detrimental effects on endophyte continues.

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Follow your dreams, no matter how far or how long it will take you to meet them, because only then you will attain personal growth. Remember, live your dreams and not someone else's....

Eduardo Sandoval



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# Chapter 1. Introduction

## 1.1. Background

Blind seed disease (BS), an infection caused by the fungus *Gloeotinia temulenta* (Prill. & Delacr.) M. Wilson, Noble & E. G. Gray 1954 (Calvert and Muskett, 1945; Wilson *et al.*, 1954) has been an important issue in the seed industry since the 1920s in different countries, such as the United States (Hardison, 1945), Australia (McGee, 1971), New Zealand (Greenall, 1943), England (Noble and Gray, 1945) among others. It affects about 56 species of grasses (Hyde, 1938). Many of the species affected are important turf and forage grasses such as *Lolium*, *Agrostis*, and *Festuca* (Alderman, 2001). BS disease affects New Zealand seed crops periodically. According to Alderman (2001), this disease is prone to occur in areas of seed production during cool seasons. It is also particularly affected by high moisture during summer, or wet weather during anthesis, and early seed fill. It is well known that *G. temulenta* directly affects the germination of ryegrass seeds (Hampton and Scott, 1981; Chynoweth *et al.*, 2012). The reduction in the incidence of the disease can be undertaken through timely use of fungicides. However, there is concern that the control of BS with fungicides may in turn cause a decrease in the transmission of desirable fungal endophytes (*Epichloë festucae* variety *lolii* syn. *Neotyphodium lolii*) to the developing seed (Harvey *et al.*, 1982). In this context, Latch and Christensen (1982), reported the use of fungicides (such as Benomyl) eliminating endophytes from infected plants.

Endophytic fungi of the genus *Epichloë* are important in pastoral agricultural systems because of their ability to increase the competitiveness of certain agronomic host grasses. This fungus is an endosymbiont that lives within a number of grass species. In particular, the agronomically important, tall fescue (*Festuca arundinacea*), and perennial ryegrass (*Lolium perenne*) have a symbiotic association (endosymbiosis) with the fungus. This asymptomatic endophyte infection provides a number of benefits to grasses. These include improved plant growth, increased resistance to invertebrate pest attack, resistance to nematodes and some fungal pathogens, decreased overgrazing, and drought tolerance. All these are contributing to the productivity of pasture in New