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# THE INCIDENCE OF ANTHRACNOSE (*COLLETOTRICHUM* SPP.) ON LUPINE SEED

Roma SEMAŠKIENĖ[1], Irena BRAZAUSKIENĖ[1], Raisa LISOVA[2], Nijolė LIEPIENĖ[1], Zita MAKNICKIENĖ[2]

[1]Lithuanian Institute of Agriculture
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania
E-mail: roma@lzi.lt

[2]Vokė Branch, Lithuanian Institute of Agriculture
Žalioji a. 2, Trakų Vokė, Vilnius distr., Lithuania

## Abstract

Lupine anthracnose is caused by the fungus of *Colletotrichum* spp. It is the most damaging disease of lupines in European and other countries. This disease is becoming increasingly common in Lithuania too. The disease spreads mainly through movement of infected seed. Seed testing is a good insurance, which will assist in the early detection of the disease. Growing of resistant varieties is a very important tool of disease control also. Seed-borne infection by anthracnose (*Colletotrichum* spp.) was investigated. The seeds of 9 lupine varieties ('Antaniai', 'Boltensija' 'Bora', 'Borlu', 'Borweta', 'Derliai' 'Trakiai', 'Ugniai' and 'Vilniai'), grown at the Vokė Branch of the Lithuanian Institute of Agriculture were analyzed. Unsterilized seeds were assayed using an incubation test. Typical symptoms of anthracnose were observed on seedling roots and hypocotyls. Analyses of seed of different varieties, obtained from pods with different anthracnose severity showed clear effect of pod infection intensity on seed health. Seedling infection level varied from low to high, and differences between the tested varieties were established.

Key words: lupine, seed infection, anthracnose.

## Introduction

Anthracnose, caused by the *Colletotrichum* spp., is a potentially devastating seed-borne disease which has become widespread in all parts of the world where lupines are cultivated and has become a major limiting factor for lupine production /Golubev, Kurlovich, 2002; Nirenberg, 2002; Talhinhas et al., 2002/. In the early 1980s, anthracnose of lupines spread in Europe /Reed et al., 1996/, as well as in Canada /Paulitz, 1995/, Australia /Sweetingham et al., 1995/. Anthracnose has been present in Belarus since 1972, but repeated epidemic developments have been recorded since 1997 /Evsikov et al., 1999, Евсиков, Иванюк, 2001 b/. The disease was first encountered in Poland in July 1995 on white lupine, grown in an experimental field /Frencel, 1998/. The first occurrence of anthracnose on lupine in Lithuania was recorded in 1997 /Maknickiene, 2001/. The disease can affect any above-ground plant part at any stage of development. However, early infections usually result in heavier yield losses and higher

seed transmission rates. Crop losses to the disease range from 10 to 100 % /Евсиков, Иванюк, 2001 a; Golubev, Kurlovich, 2002; Filoda, Horoszkiewicz-Janka, 2003; Lisova, Nedzinskienė, 2006/.

The pathogen is primarily spread by infected seed and can survive on infected crop stubble. Seedlings that emerge from infected seed can develop lesions on the roots, hypocotyls or cotyledons. Lesions are generally oval shaped, pink to beige and up to 2 cm long and cause the stem to bend and may progress to infect the pods and seeds. Infected seed also can introduce new races of the pathogen into different geographic regions. Anthracnose can move into new fields with infected seed, which gives rise to diseased seedlings that act as a source of inoculum of the anthracnose fungus that is spread to adjacent plants by splashing rain. Sowing infected seed also results in poorer emergence and reduced seedling vigour. Under wet and warm climatic conditions an infection rate of 0.001% of the seeds may lead to the loss of 30% of the harvest /Thomas et al., 1998/. Seed producers need to maintain very high standards of disease control in order to maximize yield and provide lupine producers with high quality, disease-free seed.

The study was designed to establish the impact of anthracnose pressure on pods on seed health and the influence of different varieties on seed-borne seedling infection.

**Materials and Methods**

The different varieties of lupine were grown in the experimental field of the Vokė Branch of the Lithuanian Institute of Agriculture in 2007. The seed health testing results of 9 lupine varieties (‘Antaniai’, ‘Boltensija’ ‘Bora’, ‘Borlu’, ‘Borweta’, ‘Derliai’ ‘Trakiai’, ‘Ugniai’ and ‘Vilniai’) are presented in this paper. The varieties ‘Antaniai’, ‘Derliai’ ‘Trakiai’, ‘Ugniai’ and ‘Vilniai’ are Lithuania-bred.

Before harvesting, anthracnose severity on pods was assessed in the field plots and pods with different severity were divided into 5 groups: 0 – no disease, 1–25, 26–50; 51–75 and 76–100 % area of pod affected. A total of 480 pods from each infection group was taken for the laboratory seed health analyses using an incubation test. This test is recommended by Reed et al. (1996) for lupine seed-borne infection with *Colleto-trichum* spp.

For seed health test, 100 unsterilized seed per sample were plated on well water soaked filter papers in Petri dishes (10 seeds per dish). The Petri dishes were kept in plastic bags. Moist filter paper in the plastic bag bottom was used to maintain high humidity during incubation. The test samples were incubated at 20° C temperature under 12 hours alternating cycles of near ultraviolet light (NUV) and darkness in a chamber with controlled conditions. Germinating seedlings were visually examined for anthrac-nose infection two weeks after the start of the test. Typical symptoms of anthracnose were observed on seedling roots and hypocotyls (seedling stems).

Lupines of different varieties were harvested and seeds were analysed for *Colletotrichum* spp. infection level. The incubation test was also used.

The results were statistically verified using the ANOVA modified by P. Tara-kanovas and S. Raudonius (2003). For the reliability of the data Fisher test was used. Significance was set at the 0.01 and 0.05 probability levels.

**Results and Discussion**

Analyses of different varieties' seed, obtained from the pods with different anthracnose severity showed a clear effect of pod infection intensity and variety on seed health.

Seedlings emerged from the seed of pods with no visual anthracnose symptoms were slightly infected by anthracnose, while the seedlings that emerged from the seed of heavily infected pods were affected by anthracnose significantly more severely in most cases (Figures 1 and 2). This experiment suggests that using harvested seed from diseased plants in the following season is risky, because of severe epidemics resulting in yield reduction. Anthracnose epidemics is known to generally start with a few infected seeds planted in a field /Thomas et al., 1998; Filoda, Horoszkiewicz-Janka, 2003/.

The roots of seedlings grown from the seed of healthy pods were significantly less damaged by the anthracnose after a 14-day incubation compared with the seedlings grown from infested pods' seed for all varieties. Response of different varieties to seedling root infection was recorded. The seeds from healthy pods and from those with severity ranging from 1 to 50% were less damaged for the varieties 'Antaniai', 'Borweta' and 'Derliai' compared with the those from the pods with severity higher than 50%. Seedling roots of the varieties 'Bora', 'Trakiai' and 'Ugniai' grown from uninfected pod seed, were significantly less damaged compared with those from infested pod seed. Lupine 'Boltensija' was found to be the most susceptible to seedling root infection. Although the seeds were obtained from visually healthy pods, percentage of affected seedling roots was very high.

In our laboratory experiment, the hypocotyls of different varieties were also affected by anthracnose at different levels. The hypocotyls of the varieties 'Antaniai', 'Bora', 'Borlu' and 'Borweta' were less damaged compared with those of the other varieties tested. 'Boltensija' and 'Ugniai' exhibited the highest susceptibility. None of the varieties showed complete resistance to anthracnose, even those grown from the seeds of healthy pods. However, higher seedling infection was established for all varieties grown from the seed obtained from the pods with high anthracnose severity. Our experimental evidence agrees with that obtained by other researchers, suggesting that growing an anthracnose resistant variety does not ensure that the harvested grain is free of anthracnose /Thomas, Sweetingham, 2004/.

The harvested seeds from different lupine varieties were tested. Differences in anthracnose susceptibility among the tested varieties were statistically significant after 14 days' incubation. Seedlings of 'Antaniai', 'Bora' and 'Vilniai' were more resistant to anthracnose compared to those of the other varieties tested. 'Ugniai' was mostly susceptible to this disease. Our experimental findings showed all varieties to be subject to infection by anthracnose but the infection level was highly variable.

Different prevention and control measures of seed-borne anthracnose are recommended: sowing of disease-free seed, growing of resistant varieties /Thomas, Sweetingham, 2003; Cwalina-Ambroziak, Kurowski, 2004/, fungicidal seed treatments /Horoskiewicz, Filoda, 2001; Евсиков, Иванюк, 2001 a; Thomas, Sweetingham, 2003/, exposure of lupine seed to dry heat /Thomas, Adcock, 2004/, duration of storage /Cwalina-Ambroziak, Kurowski, 2004/. In Lithuania, studies were done on the effects of different seed treatment fungicides on seed-borne anthracnose, however, the chemical seed treatment was not completely effective in eradicating the pathogen from infected seeds /Nedzinskienė et al., 2008/.

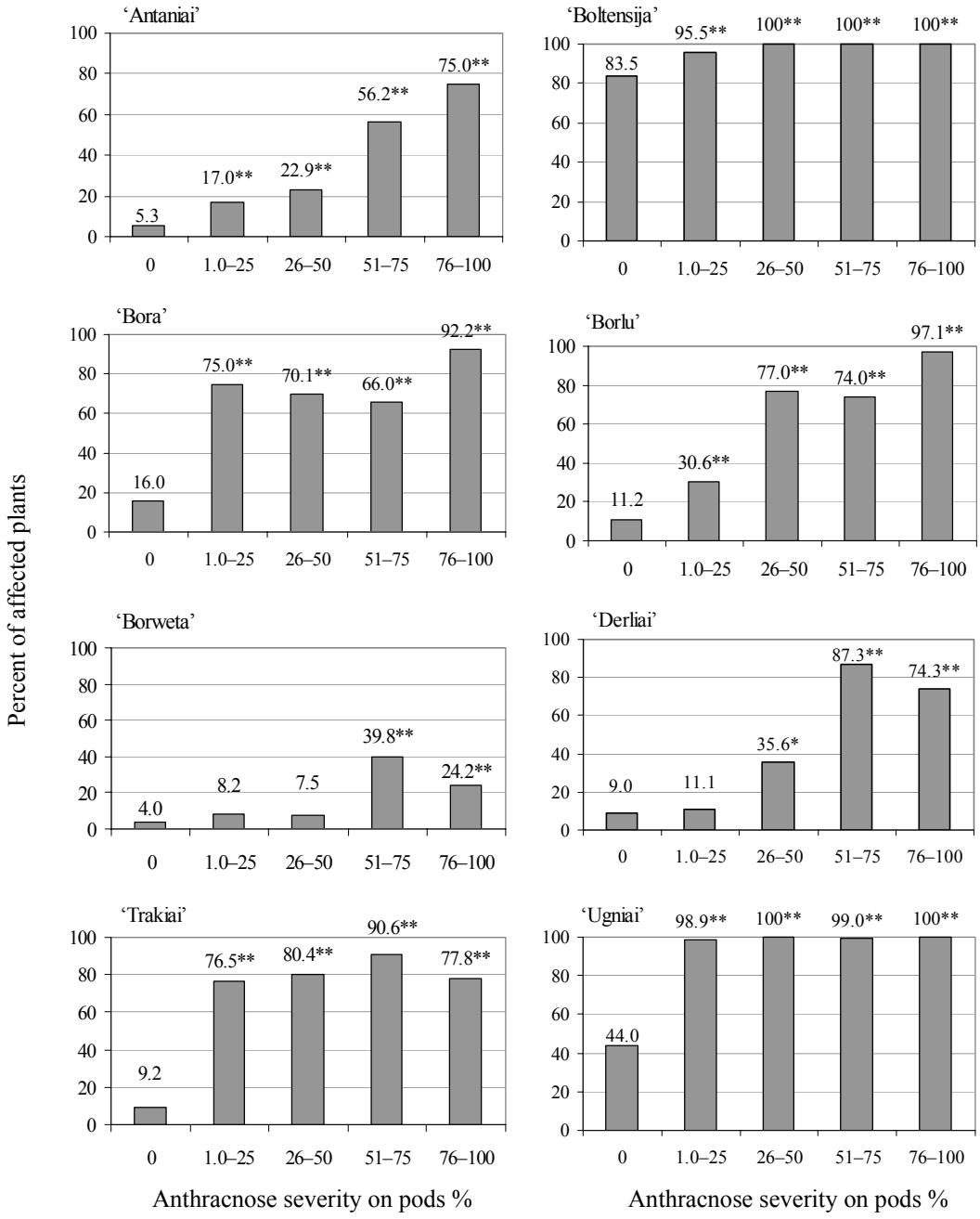


***Figure 1.*** Anthracnose incidence on seedling roots. The seeds were obtained from the pods differing in anthracnose severity (0 – no infection, 1–25, 26–50, 51–75, 76–100% disease affected area). Significant differences from the control (no infection) are marked as **(P = 0.01) and *(P = 0.05).

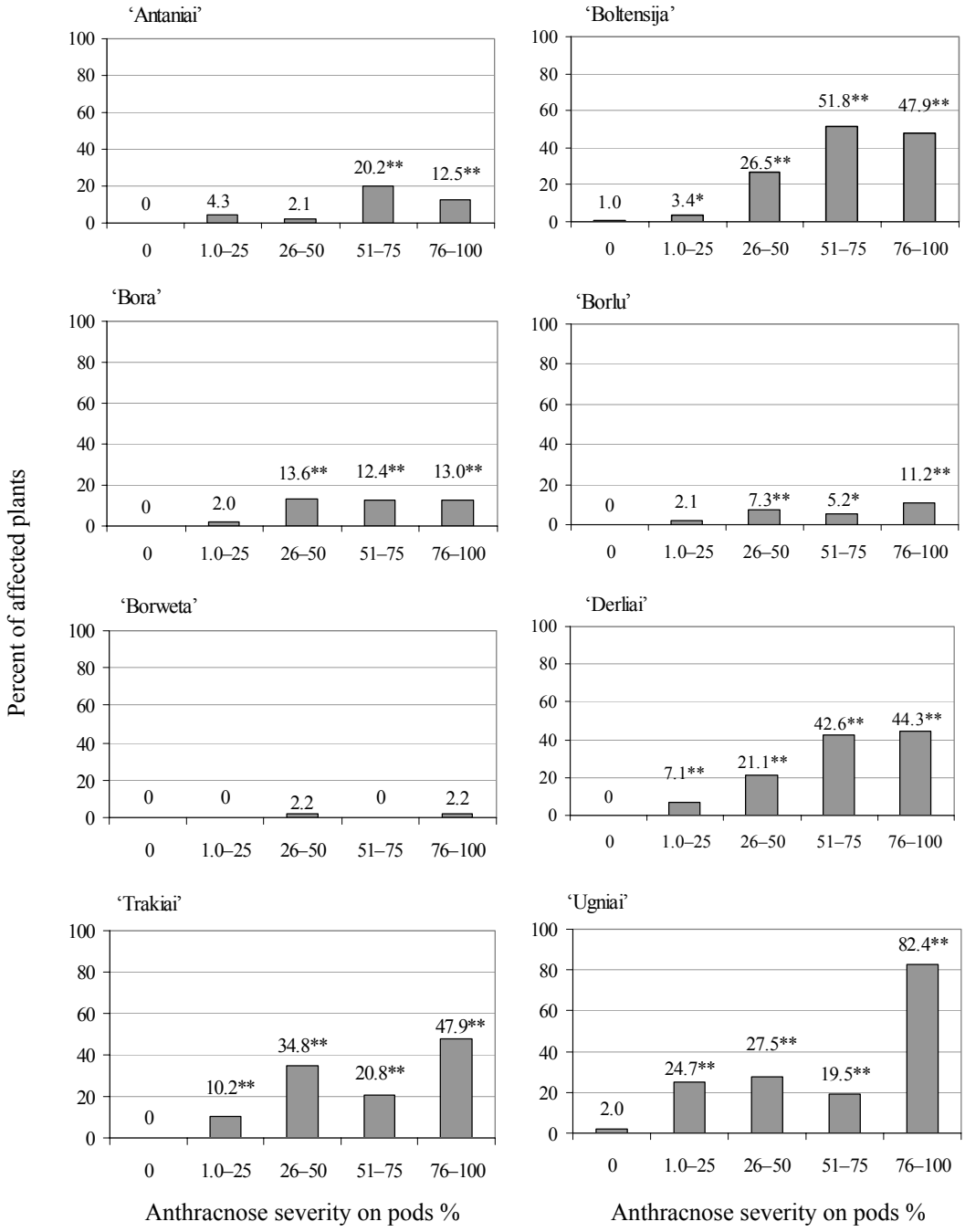


***Figure 2.*** Anthracnose incidence on hypocotyls. The seeds were obtained from the pods differing in anthracnose severity (0 – no infection, 1–25, 26–50, 51–75, 76–100% disease affected area). Significant differences from the control (no infection) are marked as **(P = 0.01) and *(P = 0.05).

***Table.*** Susceptibility of different lupine varieties to seed-borne anthracnose (incubation time 14 days)

| Variety | Anthracnose severity on seedling roots % | Anthracnose severity on hypocotyls % |
|---|---|---|
| 'Antaniai' | 45.61 abc | 16.34 abc |
| 'Bora' | 32.14 a | 18.18 abc |
| 'Borlu' | 61.89 cde | 36.06 bc |
| 'Borweta' | 61.53 bcde | 23.74 abc |
| 'Trakiai' | 54.61 abcde | 26.44 abc |
| 'Ugniai' | 83.92 e | 42.45 c |
| 'Vilniai' | 33.08 ab | 10.05 a |

Means in the same column with the same letter are not significantly different ($P<0.05$)

Our experiment showed that sowing of seeds from healthy stands can be an effective way of initial control of anthracnose. The use of disease-free seed is a critical component of any strategy to prevent losses caused by this disease. Resistance to anthracnose of different varieties is a valuable control measure /Thomas, Sweetingham, 2003; Cwalina-Ambroziak, Kurowski, 2004/. Our initial tests verify this proposition. The experiment is being continued in 2008–2009 within the programme supported by the Ministry of Agriculture of the Republic of Lithuania.

## Conclusions

1. Pod infection intensity and variety were found to be the major factors determining seed health.
2. Planting of anthracnose-free lupine seed is the primary means to limit the introduction of the pathogen into a field.
3. Seed infection level significantly differed between the lupine varieties tested, therefore in the presence of the pathogen, the best option is to use resistant varieties.

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