Susceptibility of lupin cultivars to South African isolates of *Colletotrichum gloeosporioides* associated with lupin anthracnose

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Lupin anthracnose caused by *Colletotrichum gloeosporioides* is a worldwide problem in the cultivation of lupins (*Lupinus* spp.) and has been present in South Africa since 1993. Six local isolates of the pathogen obtained from different localities were compared with an isolate collected in 1995. Fourteen-day-old seedlings of 15 lupin cultivars were spray-inoculated in the greenhouse with the seven isolates. Disease severity was rated 14 days after inoculation. No differences in virulence were detected among isolates. The most susceptible selections of *Lupinus albus* were cultivars Kiev Mutant, Ésta, Swartland and Vladimir while *Lupinus angustifolius* cultivars Wonga and Tanjil were more resistant.

Key words: anthracnose, disease resistance, lupin cultivars, South Africa.

Anthracnose is currently the most devastating fungal disease of lupins (*Lupinus* spp.) worldwide (Ageav 1993; Dick 1994; Gondran 1994; Savchenko et al. 1994; Paultiz et al. 1995; Sweetingham et al. 1995; Koch 1996; Frencel 1998; Von Baer et al. 1999). Bending or twisting of stems are characteristic disease symptoms. The stem eventually curls up and the entire plant becomes distorted. Elongated, oval, brown lesions of 0.5–2.0 cm or larger occur in the vicinity of curled-up stem sections. The centres of the lesions are creamy to pinkish-orange due to the production of masses of slimy spores. Leaves and floral parts are also attacked and round lesions are formed on the pods, from which the fungus spreads to the seed. Distinct lesions are not formed on the seed, which make it difficult to detect infection (Sweetingham 1997).

There is still confusion regarding the exact identity of the causal agent. Some claim the disease is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Gondran 1994) or *Colletotrichum acutatum* J H Simmonds (Reed et al. 1996), while others have produced evidence that it might be a separate species (Koch 1996) or formae specialis of *C. gloeosporioides* (Yang & Sweetingham 1998) or *C. acutatum* (Lardner et al. 1999; Johnston 2000). In a recent study Nirenberg et al. (2002) described two varieties, namely *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn, comb nov. var. *lupini* and *C. lupini* var. *setosum* Nirenberg, Feiler & Hagedorn var. nov. For the purpose of this study, the causal fungus will be referred to as *C. gloeosporioides*.

Currently, lupin anthracnose affects not only the production of *Lupinus angustifolius* L. (Sweetingham et al. 1995), but also *Lupinus albus* L. (Gondran 1994), *Lupinus arboreus* Sims (Dick 1994), *Lupinus luteus* L. (Sweetingham 1997), *Lupinus consentinii* Guss (Sweetingham 1997), *Lupinus mutabilis* Sweet. (Von Baer et al. 1999) and *Lupinus polyphyllus* Lind. (Reed et al. 1996). In South Africa, the disease was first recorded during the summer of 1993/1994 in the Free State Province (Koch 1996). It has since spread to the Western Cape Province, the main lupin-producing area in South Africa where lupin production has been reduced by two-thirds since the outbreak of the disease in 1996 (Koch 1999).

Studies on the epidemiology of the disease (Gondran & Pacault 1997) and an attempt by Cowling et al. (1999) to determine worldwide genetic variation in the pathogen population were undertaken in several countries, including South Africa. Despite differences in trial methodology and climatic/environmental conditions during these trials, no major differences between the various populations or isolates of the pathogen were detected.

The South African lupin industry is, for the first time during the last decade, recovering after powdery mildew devastated production in the early 1970s. However, since the outbreak of

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Table 1. Hosts, localities and collection dates of *Colletotrichum gloeosporioides* isolates.

Isolate	Host	Locality	Collection date
SHK 1033	*L. albus*	Elsenburg near Stellenbosch	1995
SHK 2140	*L. albus* cv. CEO	Hopefield	1999
SHK 2141	*L. albus*	Roodebloem near Caledon	1999
SHK 2148	*L. albus*	Langgewens near Malmesbury	1999
SHK 2153	*L. luteus* cv. Laget	Philadelphia	2000
SHK 2154	*L. albus* cv. Sonet	Roodebloem near Caledon	2000
SHK 2155	*L. albus* cv. Swartland	Roodebloem near Caledon	2000

anthracnose in 1993 (Koch 1996), unpredictable weather conditions and a decline in lupin production have made it difficult to determine possible shifts in pathogen variability under field conditions. The South African and Australian lupin industries rely on more resistant *L. angustifolius* cultivars to combat anthracnose. Any change in the virulence of *C. gloeosporioides* could therefore be devastating to lupin production in both countries.

The present study was conducted to determine the susceptibility of lupin cultivars to South Africa isolates of *C. gloeosporioides* and to establish if shifts in virulence of the pathogen have occurred since the first outbreak of disease in 1993.

Materials and methods

Collection of isolates and inoculum preparation

Diseased stem pieces or pods showing typical anthracnose lesions were collected from lupin fields in different geographic localities where the disease was present between 1999 and 2000 (Table 1). Pieces of diseased plant material were surface-disinfested for two minutes in 1 % NaOCl solution and rinsed three times with sterile distilled water. Small pieces of tissue from the surface (2 mm^3) were incubated on potato-dextrose agar (PDA) supplemented with 50 mg l^{-1} streptomycin and chloramphenicol. Single-spore isolates were prepared from cultures identified as causing lupin anthracnose and maintained on potato carrot agar (PCA) slants at 5 °C.

Two sets of isolates (SHK 2141, SHK 2148 and SHK 2140, and SHK 2153, SHK 2154 and SHK 2155) (Table 2) were used to inoculate plants in two separate trials. Isolate SHK 1033, collected in 1995 in the Western Cape and maintained on PCA slants at 5 °C, was included in both trials as a reference. The isolates were cultured on PDA at 23 °C under a 12-hour light/darkness regime. Conidia were dislodged in sterile distilled water and the conidial suspension was adjusted using a haemocytometer to 5×10^6 conidia ml^{-1}. Two drops of Tween 20 were added to the conidial suspension to reduce the surface tension on leaves and run-off.

Soil preparation and planting

Sixteen lupin accessions with different genetic backgrounds (Table 2), some of which are currently being evaluated in the National Lupin Cultivar Trials, were selected. Lupin seeds were surface-disinfested for 10 minutes in a 1 % NaOCl solution and rinsed three times with sterile distilled water. Eight seeds per cultivar were planted in a seedling tray with 128 wells, each 3 × 3 × 10 cm in size, containing a 1:1 mixture of virgin loam soil and perlite (Genolite). Seeds were covered with a 5 mm layer of river sand. For each experiment, a split plot design with isolates as the main plot and cultivars as subplot was employed. Each treatment was replicated four times. The seedlings were cultivated for 14 days in a greenhouse at 20 ± 2 °C and were watered regularly. Prior to inoculation, plants were watered thoroughly to maintain moisture levels for seven days.

Inoculation

When 14 days old, seedlings were sprayed until run-off with the respective conidial suspensions and the controls with sterile distilled water. Each tray was covered with transparent plastic sheeting to maintain humidity close to 100 %. Treatments were separated by means of plastic dividers to prevent cross-contamination. To further simulate conditions conducive to disease development, the greenhouse roof was covered with 80 % shade cloth (Alnet™). Within the greenhouse, an additional layer of 50 % shade cloth was fitted 1 m above the seedlings to provide additional protection from direct sunlight. After five days, holes of approximately 4 cm^2 were cut in the plastic cover-