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Relative susceptibilities to Sphaeropsis sapinea of six Pinus spp. cultivated in South Africa

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Abstract

The relative susceptibilities to *Sphaeropsis sapinea* of six *Pinus* spp. cultivated in South Africa were investigated in growth chamber and field inoculations. The relative susceptibilities of the species tested, compare favourably with field observations by foresters over a number of years. There was some variation in pathogenicity among *S. sapinea* isolates on the different species.

1 Introduction

Sphaeropsis sapinea (Fr.) Dyko et Sutton [*Diplodia pinea* (Desm.) Kickx] is a pathogen on over 33 *Pinus* species throughout the world (Gibson 1979; Punithalingham and Waterston 1970; Swart, Knox-Davies and Wingfield 1985; Waterman 1943). It causes shoot blight, cankers, collar rot, root disease and bluestain (Brookhouser and Peterson 1971; Chou 1976 a, b; Eldrige 1957; Gibson 1979; Laughton 1937; Marks and Minko 1969, 1970; Swart, Knox-Davies and Wingfield 1985; Waterman 1943; Wingfield and Knox-Davies 1980 a, b).

The most practical control for diseases caused by *S. sapinea* is to plant resistant pine species (Burdon, Currie and Chou 1980; Gibson 1979; Lückhoff 1964). However, the selection of pines to be used in commercial afforestation in Southern Africa is based on species and provenance trials which concentrate on climatic and edaphic factors that influence growth (Poynton 1979). No evaluations are made for disease resistance. Current knowledge of the relative susceptibilities of different pine species to *S. sapinea* is based solely on field observations by foresters.

The major commercial pines grown in Southern Africa are: *Pinus patula* Schlect. et Cham., *P. taeda* L., *P. elliottii* Engelm. et Vasey and *P. kesiya* Royle ex Gord, in the summer rainfall region, and *P. pinaster* Ait. and *P. radiata* D. Don in the constant and winter rainfall regions (Poynton 1979). In this study we evaluated the resistance of the above species to *S. sapinea* in growth chambers and the forest.

2 Materials and methods

2.1 Growth chamber inoculations

The following seedlots were obtained from the South African Forestry Research Institute, Pretoria: *P. patula* (33747), *P. radiata* (26066), *P. pinaster* (25097), *P. elliottii* (28188), *P. taeda* (28467) and *P. kesiya* (29204). Seedlings were raised on steam-sterilized soil in plastic sleeves. Forty seedlings of each species were preconditioned (Chou 1982) 5 weeks

prior to inoculation by transferring them to a growth chamber maintained at 22–25 C and 75 % relative humidity with cool white fluorescent lighting (12 hr/day). Seedlings were inoculated in February when they were 12-months old. Inoculation was repeated in July with 17-month-old seedlings from the same planting after they had been subjected to a 5-week preconditioning in the growth chamber.

A local single-spore isolate of *S. sapinea* (PREM 48859) was grown on 2 % water agar plates covered with autoclaved *P. radiata* pine needles and incubated at 25 °C under near ultra-violet light (12 hr/day) for 3 weeks. Needles bearing pycnidia were then agitated in a 0,5 % gelatin solution at 10°C for 30 min. After removing needles, the suspension was adjusted to 1×10^4–$1{,}5\times10^4$ conidia/ml.

Seedlings were wounded by puncturing the terminal shoot, 25 mm from the tip and ca. 3 mm deep, with three 12-gauge hypodermic needles set 5 mm apart. Both wounded and unwounded shoots were inoculated using a soft camel-hair brush to apply inoculum to the shoots (CHOU 1976 b). During inoculation, the conidial suspension was kept in an ice bath to prevent premature spore germination. Control plants were similarly inoculated with a sterile, 0,5 % gelatin solution. After inoculation, all plants were covered for 48 hours with clear plastic bags and held in the growth chamber. The number of seedlings with dead shoots was recorded after 3 weeks and isolations were made from all seedlings with disease symptoms.

2.2 Field inoculations

In February 1986, 4-yr-old *P. radiata, P. pinaster* and *P. elliottii* trees were inoculated at Grabouw State Forest in the South-Western Cape. Twenty trees of each species were used for each of the following inoculation procedures:

2.2.1 Shoot tip inoculations

Two pairs of shoots were chosen on each tree. One shoot in each pair was wounded as described for seedling inoculations. One pair was inoculated with a spore suspension of PREM 48859 (1×10^4–$1{,}5\times10^4$ conidia/ml in 0,5 % gelatin solution) using the camel-hair brush technique (CHOU 1976 b), and the control pair with a sterile, 0,5 % gelatin solution.

Shoots were covered with clear plastic bags for 24 hours after inoculation. The number of dead shoots was recorded after 6 months and isolations were made from all shoots with disease symptoms.

2.2.2 Branch inoculations

Trees were wounded on each of four lateral branches by removing cambial discs with an 8 mm diameter cork borer. A disc from each of three, 4-day-old single-conidial cultures of local *S. sapinea* isolates (PREM 48859, PREM 48860 and PREM 48892; National Collection of fungi, Pretoria, South Africa) grown on 1 % Difco malt-extract agar (MEA) was placed in each of three wounds per tree. A sterile MEA disc was placed in the fourth wound. Wounds were covered with Parafilm^R. After 6 months, bark and phloem surrounding inoculation points were removed with a scalpel and the length of discoloured cambium measured and isolations were made from discoloured tissue. A one-way analysis of variance (ANOVA) was performed and Student's least significant differences (LSD) were calculated to separate treatment means between pine species (Table 2). A two-way ANOVA was performed and LSD were calculated to separate treatment means between *S. sapinea* isolates (Table 2).

3 Results

3.1 Growth chamber inoculations

Both trials had more dead shoots in wounded than in unwounded seedlings (Table 1) and *S. sapinea* was isolated from all dead shoots. All control plants remained symptomless.

In trials with 12- or 17-mo-old seedlings, both wounded and unwounded *P. radiata*, *P. pinaster* and *P. kesiya* plants were significantly more susceptible (P = 0.05) than *P. elliottii*, *P. patula* and *P. taeda*. *P. taeda* seedlings that had not been wounded prior to inoculation with *S. sapinea* were completely unaffected in both trials.

3.2 Field inoculations

3.2.1 Shoot tip inoculations

No infection was apparent in any of the controls or unwounded shoots inoculated with *S. sapinea* (Table 1). After wounding, *P. radiata* was significantly more susceptible (P = 0.05) than *P. pinaster* or *P. elliottii* (Table 1).

Table 1. **Susceptibility (% dead shoots) of six Pinus spp. to S. sapinea following growth chamber and field inoculations[1]**

Pinus sp.	Growth chamber inoculations[2]				Field inoculations[3]	
	12-months-old		17-months-old			
	WI	NWI	WI	NWI	WI	NWI
P. elliottii	70	10	70	20	35	0
P. kesiya	100*[4]	90*	90*	40*	–	–
P. patula	60	20	60	30	–	–
P. pinaster	100*	50*	80*	40*	45	0
P. radiata	80*	70*	100*	80*	80*	0
P. taeda	50	0	40	0	–	–

[1] Treatments: WI = wounded; NWI = non-wounded.
[2] 10 seedlings inoculated for each treatment.
[3] 20 shoots of 4-yr-old trees inoculated in each treatment.
[4] Values within columns followed by * represent the 'best group' (P = 0.05) according to Gupta's t-test procedure (Gupta and Panchapakeson 1979).

Table 2. **Mean length of cambial lesions (mm) on three Pinus spp. following inoculations with three isolates of S. sapinea**

S. sapinea isolate	*Pinus* sp.			LSD[x]
	P. radiata	*P. pinaster*	*P. elliottii*	
PREM 48859	31.65 a1	25.90 a12	23.35 a2	5.95
PREM 48860	26.65 b1	18.45 b2	16.80 b2	4.24
PREM 48892	28.25 ab1	23.60 a1	16.70 b2	4.94
Control	12.90 c1	10.45 c2	10.85 c2	1.40
LSD[y]	3.78	3.98	2.90	

[x] One way ANOVA. Values followed by the same number between columns are not significantly different (P = 0.05).
[y] Two way ANOVA. Values followed by the same letter within columns are not significantly different (P = 0.05).

3.2.2 Branch inoculations

S. sapinea was consistently isolated from all discoloured tissue. The lengths of cambial discolouration associated with all isolate/host combinations was significantly greater ($P = 0.05$) than that in the controls (Table 2).

Cambial lesions were significantly longer ($P = 0.05$) on *P. radiata* than on *P. elliottii* for each of the three *S. sapinea* isolates tested (Table 2). Significant differences ($P = 0.05$) in lesion length between *P. radiata* and *P. pinaster*, and *P. pinaster* and *P. elliottii*, occurred only with isolates PREM 48860 and PREM 48892 respectively. Cambial lesions caused by isolate PREM 48859 on each of the three pine species were significantly longer ($P = 0.05$) than lesions caused by PREM 48860 (Table 2).

4 Discussion

The relative susceptibilities of the major local commercial pines to *S. sapinea*, as indicated in this study, compare favourably with field observations by foresters (POYNTON 1979) over a number of years. *P. radiata* and *P. pinaster* are generally regarded as being more susceptible to *S. sapinea* than *P. elliottii*, *P. patula* and *P. taeda* (BROWN et al. 1981; BEGA et al. 1978; GIBSON 1979; LAUGHTON 1937; PUNITHALINGAM and WATERSTON 1970; VAN DER WESTHUIZEN 1968; WATERMAN 1943). Caution should, however, be exercised when making comparisons, as differences in host genotype, pathogen isolate, environmental conditions and season can influence susceptibility (CHOU 1982; YARDWOOD 1959).

P. radiata is reputed to be more susceptible to *S. sapinea* than *P. pinaster* (GIBSON 1979; PUNITHALINGAM and WATERSTON 1970; WATERMAN 1943). Although our growth chamber inoculations did not indicate significant differences in susceptibility between these species, results of field inoculations indicated that *P. radiata* was significantly more susceptible. *P. kesiya* is reported to be almost completely free from diseases and pests and because of its excellent timber has been commended for use on an increased scale in Southern Africa (POYNTON 1979). The results of our study, however, show that *P. kesiya* is as susceptible to *S. sapinea* under growth chamber conditions as *P. radiata* and *P. pinaster*. *P. kesiya* is not cultivated in the South-Western Cape and its susceptibility in the forest was therefore not determined.

The relative susceptibilities of *P. patula*, *P. elliottii* and *P. taeda* cannot be clearly evaluated from the literature. GIBSON (1979) found *P. patula* to be more resistant to *S. sapinea* than either *P. taeda* or *P. elliottii*. This is contrary to reports by LÜCKHOFF (1964) and POYNTON (1979) that, in the worst hail belts of South Africa, *P. patula* is being replaced as far as possible with *P. elliottii* which is generally considered to be the more resistant species. Although our results show no significant differences in susceptibility between these species, *P. taeda* had the lowest mortality rates of any of the six species tested. *P. patula* and *P. taeda* are also not grown in the South-Western Cape and could not be included in field studies.

Variation in pathogenicity among isolates of *S. sapinea* has been reported in the United States (PALMER et al. 1987; WANG et al. 1985) and in South Africa (SWART et al. 1987). These observations, together with results of the present study, emphasize the importance of using, where possible, several different isolates in inoculations.

Growth chamber inoculations provide a convenient and relatively effective way of evaluating the interspecific resistance of pines to *S. sapinea*. However, in this investigation field inoculations gave more distinct results. Further studies are needed to evaluate fully the relative susceptibilities to *S. sapinea* of *P. radiata*, *P. pinaster* and *P. kesiya* on the one hand and *P. elliottii*, *P. patula* and *P. taeda* on the other.

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Summary

Wounded and unwounded shoots of *Pinus elliottii, P. patula* and *P. taeda* seedlings inoculated with *Sphaeropsis sapinea* in a growth chamber had significantly less dead shoots after three weeks than *P. radiata, P. kesiya* and *P. pinaster*. Wounded shoots of 4-yr-old *P. pinaster* and *P. elliottii* trees inoculated with *S. sapinea* had significantly less dead shoots than *P. radiata*. Branches of 4-yr-old *P. radiata* trees inoculated with three *S. sapinea* isolates had significantly longer cambial lesions than *P. pinaster* for one isolate and *P. elliottii* for three isolates.

Résumé

Sensibilité relative à Sphaeropsis sapinea, de six espèces de pins cultivées en Afrique du Sud

Des pousses blessées et non blessées de semis de *Pinus elliottii, P. patula* et *P. taeda*, inoculées en chambre climatisée par *Sphaeropsis sapinea* subissaient significativement moins de mortalité après trois semaines comparativement à *P. radiata, P. kesiya* et *P. pinaster*. Chez des plants de quatre ans, les pousses blessées puis inoculées de *P. pinaster* et *P. elliottii* présentaient significativement moins de mortalité que celles de *P. radiata*. Les lésions du cambium sur des branches inoculées de *P. radiata* de 4 ans étaient significativement plus longues que celles obtenues sur *P. pinaster* pour un isolat, et sur *P. elliottii* pour trois isolats.

Zusammenfassung

Relative Anfälligkeit von sechs in Südafrika angebauten Pinus-Arten gegenüber Sphaeropsis sapinea

Verletzte und unverletzte mit *Sphaeropsis sapinea* in einer Klimakammer inokulierte Triebe von *P. elliottii-, P. patula-* and *P. taeda*-Sämlingen hatten nach drei Wochen weniger Abgänge als die von *Pinus radiata, P. kesiya* und *P. pinaster*. Verletzte Triebe von vierjährigen *P. pinaster* und *P. elliottii* starben nach *S. sapinea*-Inokolation gesichert weniger häufig ab als bei *P. radiata*. Mit drei *S. sapinea*-Isolaten inokulierte Zweige vierjähriger *P. radiata* hatten signifikant längere Kambium-Läsionen als *P. pinaster*-Inokulationen mit einem, und *P. elliottii*-Inokulationen mit drei Isolaten.

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