Phytophylactica 19, 505–510 (1987)

FACTORS ASSOCIATED WITH *SPHAEROPSIS SAPINEA* INFECTION OF PINE TREES IN SOUTH AFRICA*

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ABSTRACT

Key words: *Diplodia pinea*, *Pinus* spp., *Sphaeropsis sapinea*

Postal and field surveys were used to study the occurrence of *Sphaeropsis sapinea* on *Pinus* spp. in southern Africa during 1985 and 1986. Thirty-nine cases of disease were recorded. The most common symptoms were shoot blight and dead top. They were usually associated with factors such as drought stress, hail damage, insect damage or nutrient stress. Shoot blight and dead top also occurred in the absence of *S. sapinea*. Variation in cultural characteristics was observed among isolates of the fungus. Two isolates were compared. They differed significantly in spore size and in their growth rates at different temperatures and on different media. One isolate was significantly more virulent in stem and shoot inoculations.

Uittreksel

FAKTORE GEASSOSIEER MET SPHAEROPSIS SAPINEA-*INFEKSIE VAN DENNEBOME IN SUID-AFRIKA*

Pos en veldopnames om die voorkoms van Sphaeropsis sapinea *op* Pinus *spp. gedurende 1985 en 1986 te bestudeer is gedoen. Nege-en-dertig gevalle van siekte is aangeteken. Die mees algemene simptome was lootskroei en dooie toppe. Albei was gewoonlik met faktore soos droogte, haelskade, insekskade of voedingstekorte geassosieer. Lootskroei en dooie toppe het ook in die afwesigheid van* S. sapinea *voorgekom. Variasie in kultuureienskappe van isolate van die swam is waargeneem. Twee isolate is met mekaar vergelyk. Verskille in spoorgrootte en in groeitempo by verskillende temperature en op verskillende media was betekenisvol. Een isolaat was meer virulent as die ander.*

INTRODUCTION

Sphaeropsis sapinea (Fr.) Dyko & Sutton [*Diplodia pinea* (Desm.) Kickx] has caused severe damage to *Pinus* spp. throughout the world (Gibson, 1979; Punithalingam & Waterston, 1970) and is considered the most important pathogen of exotic pines in South Africa (Kotzé, 1935; Laughton, 1937; Lückhoff, 1964; Poynton, 1979; Wingfield & Knox-Davies, 1980a).

The most common symptom of *S. sapinea* infection is shoot blight (Laughton, 1937; Haddow & Newman, 1942; Waterman, 1943a, b; Van der Westhuizen, 1955; Eldridge, 1957; Millikan & Anderson, 1957; Buchanan, 1963; Gibson, 1979; Wingfield & Knox-Davies, 1980a), but canker accompanied by resinosis (Waterman, 1943b; Gilmour, 1964; Marks & Minko, 1969; Wright & Marks, 1970), bluestain (Da Costa, 1955; Eldridge, 1957), and root disease (Wingfield & Knox-Davies, 1980b) are also associated with infection.

Opinions differ as to the pathogenicity of *S. sapinea*. Infection is more common in trees stressed by drought, poor site conditions and nutrient deficiencies (Van der Byl, 1933; Laughton, 1937; Ludbrook & White, 1940; Minko & Marks, 1973; Lückhoff, 1964; Stahl, 1968; Bega *et al.*, 1978; Evans, 1978; Wingfield & Knox-Davies, 1980a; Brown, Bevege & Stevens, 1981; Bachi & Peterson, 1985), although unstressed trees are known to have been severely affected by *S. sapinea* (Eldridge, 1957; Marks, Grose & Minko, 1966; Marks & Minko, 1969, 1970; Wright & Marks, 1970). Mechanical wounding of host tissues is usually necessary for infection (Haddow & Newman, 1942; Rawlings, 1955; Purnell, 1957), but unwounded new shoots are also infected under certain climatic conditions (Laughton, 1937; Waterman, 1943b; Eldridge, 1957; Marks *et al.*, 1966; Peterson & Wysong, 1968; Brookhouser & Peterson, 1971; Chou, 1976a, b; Nicholls & Ostry, 1977; Bega *et al.*, 1978; Wingfield & Knox-Davies, 1980a; Johnson & Peterson, 1985; Palmer, 1985). The ability of *S. sapinea* to infect shoots and the resulting damage is reportedly also related to age of the tree and maturity of the host tissue (Peterson & Wysong, 1968; Chou, 1976b, 1977).

Gibson (1979) suggested that the occurrence of different strains of *S. sapinea* could explain conflicting reports of pathogenicity. Chou (1976b) could find no evidence of variation in virulence or visible differences in cultural characteristics among New Zealand isolates of *S. sapinea*. Reports from other sources (Laughton, 1937; Bachi & Peterson, 1982; Palmer & Stewart, 1982; Palmer, 1985; Wang *et al.*, 1985; Palmer, Stewart & Wingfield, 1986) support the view that there is variation in the fungus.

The present paper reports on postal and field surveys which were conducted to investigate the occurrence of *S. sapinea* diseases in South Africa and Lesotho, and the role of predisposition in infection. Preliminary studies were also made on the virulence of two isolates showing marked differences in culture.

MATERIALS AND METHODS

Postal survey

Questionnaires designed to gather information on the incidence of *S. sapinea* in South Africa and Lesotho during 1985 and 1986 were sent to 200 randomly chosen timber growers. An identification guide (Wingfield, 1980) was enclosed with each questionnaire.

Field surveys

Pine plantations in the Western, Southern and Eastern Cape Province, Natal, Transvaal and Lesotho were visited either routinely or in response to requests by foresters. The presence of *S. sapinea* in material showing disease symptoms was confirmed by isolation on malt extract agar (MEA) (20 g Difco Bacto agar, 10 g Difco malt extract, 1 dm^3 H_2O). Isolates were kept at 25 °C on MEA slants.

Isolates

Source. Two isolates (PREM 48859 and PREM 48860) which differed most in cultural appearance were selected for further study. PREM 48859 came from a *P.*

* Part of an M.Sc. (Agric.) thesis submitted by the first author to the University of Stellenbosch, Stellenbosch 7600

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Received 26 January 1987; accepted for publication 28 July 1987

TABLE 1 Incidence of *S. sapinea* associated symptoms recorded in South Africa between January 1985 and June 1986

Region	Location	Symptoms	Species	Age (yr)	Compartment No. and extent of damage[a]	Associated factors[b]
E. Tvl	Blairmore State Forest (SF)	Shoot blight, dead top	*P. patula*	4	E2b—25 trees	AB
E. Tvl	Blairmore SF	Shoot blight, dead top	*P. patula*	16	E2c—5 trees	AB
E. Tvl	Belfast SF	Shoot blight, dead top, resinosis	*P. patula*	5	C26—80 %	B
E. Tvl	Jessievale SF	Shoot blight, dead top	*P. radiata*	5	E51—25–50 %	AB
E. Tvl	Jessievale SF	Dead top	*P. patula*	5–11	F65—5–25 %	AB
Lesotho	Leshoboro plateau	Shoot blight, dead top, canker	*P. radiata*	9	5r—50–100 %	AB
Lesotho	Leshoboro plateau	Dead top	*P. radiata*	2	L/25/115FB—5–25 %	AB
Lesotho	Leshoboro plateau	Dead top	*P. radiata*	5	7c—5–25 %	AB
Lesotho	Leshoboro plateau	Dead top	*P. radiata*	2	L/25/60A—5–25 %	AB
Lesotho	Leshoboro plateau	Dead top, chlorosis	*P. radiata*	14	2c—50–100 %	AB
Lesotho	Leshoboro plateau	Dead top, chlorosis	*P. pinaster*	11	3a—25–50 %	AB
Lesotho	Leshoboro plateau	Shoot blight, dead top	*P. eldarica*	4	L/25/96—50–100 %	AB
Lesotho	Leshoboro plateau	Chlorosis	*P. halepensis*	2	6a—50–100 %	AB
Natal	E. Shores SF	Dead top	*P. elliottii*	5–15	Throughout—5–10 %	A
Natal	E. Shores SF	Shoot blight	*P. elliottii*	18	Block G—10 %	A
Natal	Farm near Ixopo	Shoot blight, dead top	*P. elliottii*	5–15	*c.* 500 trees	AB
E. Cape	Qacu SF	Resinosis	*P. radiata*	9	D9—5–25 %	AB
E. Cape	Isidenge SF	Dead top	*P. canarienis*	30	D28a—25–50 %	A
E. Cape	Isidenge SF	Shoot blight, chlorosis	*P. elliottii*	9	D1—5–25 %	A
S. Cape	Kromrivier SF	Shoot blight, dead top	*P. radiata, P. pinaster*	6–8	Block A—5–25 %	B
S. Cape	Kromrivier SF	Shoot blight, dead top	*P. radiata, P. pinster*	8–12	Block A—5–25 %	B
S. Cape	Otterford SF	Shoot blight	*P. radiata*	5–20	Throughout—5—25 %	—
S. Cape	Kruisfontein SF	Dead top	*P. radiata*	46	H8b—5–25 %	A
S. Cape	Kruisfontein SF	Shoot blight, dead top	*P. radiata*	—	Block D—10–40 %	AB
S. Cape	Bluelilliesbush SF	Shoot blight	*P. pinaster*	7	C78—10 %	A
S. Cape	Lottering SF	Shoot blight, chlorosis	*P. taeda*	28	A1c—20 %	A
S. Cape	Lottering nursery	Shoot blight	*P. radiata*	6 mths	15 %	AB
S. Cape	George district	Shoot blight, dead top	*P. radiata, P. pinaster*	5–15	943,7 ha	AB
S. Cape	George district	Shoot blight, dead top	*P. radiata, P. pinaster*	15–35	349,8 ha	AB
S. Cape	Witfontein SF	Dead top	*P. radiata*	3	Compt unknown (5–10 %)	C
S. Cape	Farm near Knysna	Shoot blight, dead top	*P. radiata, P. pinaster*	5–10	Throughout—5–25 %	—
S. Cape	Kleinberg (Mossel Bay Municipality)	Shoot blight, dead top, resinosis	*P. radiata*	30	2—*c.* 120 trees	—
S.W. Cape	La Motte SF	Dead top, canker	*P. radiata*	8–15	Throughout—5–25 %	C
S.W. Cape	Garcia SF	Shoot blight, resinosis	*P. radiata*	10–24	C10c—5–25 %	A
S.W. Cape	Swellendam, Garcia, Grootvadersbosch SF's	Shoot blight	*P. radiata*	2–15	1 513 ha—20–40 %	A
S.W. Cape	Jonkershoek SF	Shoot blight	*P. radiata*	NR[c]	Throughout—10–20 %	—
S.W. Cape	Grabouw SF	Shoot blight	*P. radiata, P. pinaster*	NR	Throughout—10–20 %	—
S.W. Cape	Grabouw SF	Dead top	*P. radiata*	10–15	Throughout—10–20 %	D
S.W. Cape	Lebanon SF	Shoot blight	*P. radiata*	NR	Throughout—10–20 %	—

[a] Presence of at least one dead shoot/tree excluding terminal shoot

[b] Associated factors: A = Occurrence of drought not more than 2 yr prior to date of observation; B = Occurrence of hail not more than 6 mo prior to date of observation; C = Insect damage; D = Nutrient deficiency

[c] Natural regeneration

TABLE 2 Growth of two isolates of *Sphaeropsis sapinea* at different temperatures and on different media after 96 h

Isolate	Colony diameter (mm)[a]										
	Temperature						Medium				
	10	15	20	25	30	35	MEA	PDA	CMA	CD	WA
PREM 48859	17,8	19,7	74,1	83,3	82,8	10,0	76,4	78,4	83,0	79,0	49,4
PREM 48860	13,9	13,4	40,4	53,5	51,0	8,6	42,4	44,8	49,0	25,4	48,0

[a] Values are means of two perpendicular measurements of colony diameter from five replicates of each treatment

TABLE 3 Pathogenicity of two isolates of *Sphaeropsis sapinea* to *Pinus radiata*

Isolate	Stem incoulation			Shoot inoculation	
	Lesion length (mm)[a]	Range	S.E.	Technique	Seedlings with dead shoots[b] (%)
PREM 48859	27,5	10–44	9,75	Wounded	80
				Not wounded	50
PREM 48860	13,2	8–22	3,96	Wounded	40
				Not wounded	10
Control	8,5	0–10	2,06	Wounded	0
				Not wounded	0

[a] Values are means of ten replicates of each treatment

[b] Ten seedlings were inoculated in each treatment

radiata shoot from Jonkershoek, Stellenbosch and PREM 48860 from a *P. taeda* root from Lottering State Forest, Knysna. Cultures of these isolates were deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Spore dimensions. The lengths and widths of 100 spores of each isolate were measured.

Growth on different media. A 5 mm plug of each isolate from an actively growing MEA culture was placed in the centre of a 90 mm petri plate containing 25 cm^3 of the following media: Difco potato-dextrose agar (PDA), MEA, Merck Czapek-Dox agar (CD), water agar (WA) (20 g Difco Bacto agar, 1 dm^3 H_2O) and Difco corn-meal agar (CMA). Plates were sealed with Parafilm and incubated in the dark at 25 °C. Colony diameters were measured along two perpendicular lines after 96 h. Culture morphology was observed after 14 d.

Growth at different temperatures. Petri plates containing 15 cm^3 MEA were inoculated as above and incubated at 10, 15, 20, 25, 30 and 35 °C. Colony diameters were measured after 96 h.

Stem inoculations. Three-yr-old *P. radiata* saplings approximately 1 m in height were inoculated by removing cambial discs from the stems with a 5 mm diameter cork borer, replacing them with discs from 3-d-old MEA cultures and wrapping the inoculation points with Parafilm. Control inoculations were conducted with sterile MEA discs. After 9 wk the surrounding bark was removed and the length of discolouration measured. Isolations were made from discoloured tissue to verify the presence of *S. sapinea*.

Shoot tip inoculations. Both wounded and unwounded growing tips of *P. radiata* seedlings were inoculated with spores using a soft camel-hair brush according to the method of Chou (1976b). Spores were produced on autoclaved pine needles placed on 2 % WA and irradiated at near ultra-violet light for 8 h/d. After 3 wk, pycnidia-bearing needles from 4-wk-old cultures were agitated in a 0,5 % gelatin solution at 10 °C for 30 min. Needles were removed and the suspension was adjusted to 1×10^4–$1{,}5 \times 10^4$ spores/cm^3. Wounds were made by puncturing the terminal portion of each shoot with three 12-gauge hypodermic needles set 5 mm apart. Control plants were inoculated with a sterile 0,5 % gelatin solution. Seedlings were incubated in clear plastic bags for 48 h and kept in a growth chamber maintained at 22–25 °C and 75 % relative humidity with cool white fluorescent lighting on a 12 h cycle for 3 wk, after which the number of seedlings with dead shoots was recorded. Isolations were made from seedlings showing disease symptoms.

RESULTS

Surveys

Thirty-nine cases of disease were documented (Table 1).

Root disease. A 20 % mortality of 28-yr-old *P. taeda* trees at Lottering State Forest (Table 1) was attributed to *S. sapinea* following isolation of the fungus from the roots. Rainfall for this area was said to have been 37 % less than in the previous year. In Lesotho, on the Leshoboro plateau, *S. sapinea* was also isolated from roots of some 20-yr-old *P. radiata* trees showing general chlorosis.

Diseases of above-ground parts. Death of lateral shoots (shoot blight) and terminal shoots (dead top) were the most common symptoms observed. In most cases they were associated with *S. sapinea* infection and factors such as drought stress, hail damage, insect damage or nutrient stress. Disease incidence was notably higher on trees under the age of 15 yr. Shoot blight and dead top also occurred sporadically in healthy trees of all ages, especially natural regeneration under mature pine stands, where stress or wounding were apparently not involved.

Hail damage was associated with 51 % and drought with 66 % of the cases of shoot blight, dead top, or resinosis. Hail in the absence of drought was associated with 7 % and drought in the absence of hail with 23 % of cases of shoot blight and dead top.

The most severe case of *S. sapinea* infection following hail damage in the absence of moisture stress was at Kromrivier State Forest near Humansdorp. Approximately 400 ha consisting largely of *P. radiata*, but also *P. pinaster*, (6 to 12-yr-old) had to be clearfelled following a hailstorm in March 1985. Isolation from hail wounds (Fig. 1, 2) consistently yielded *S. sapinea*.

In the Outeniqua district, 1 292 ha of a total area of 15 500 ha comprising mostly *P. radiata*, but also *P.*

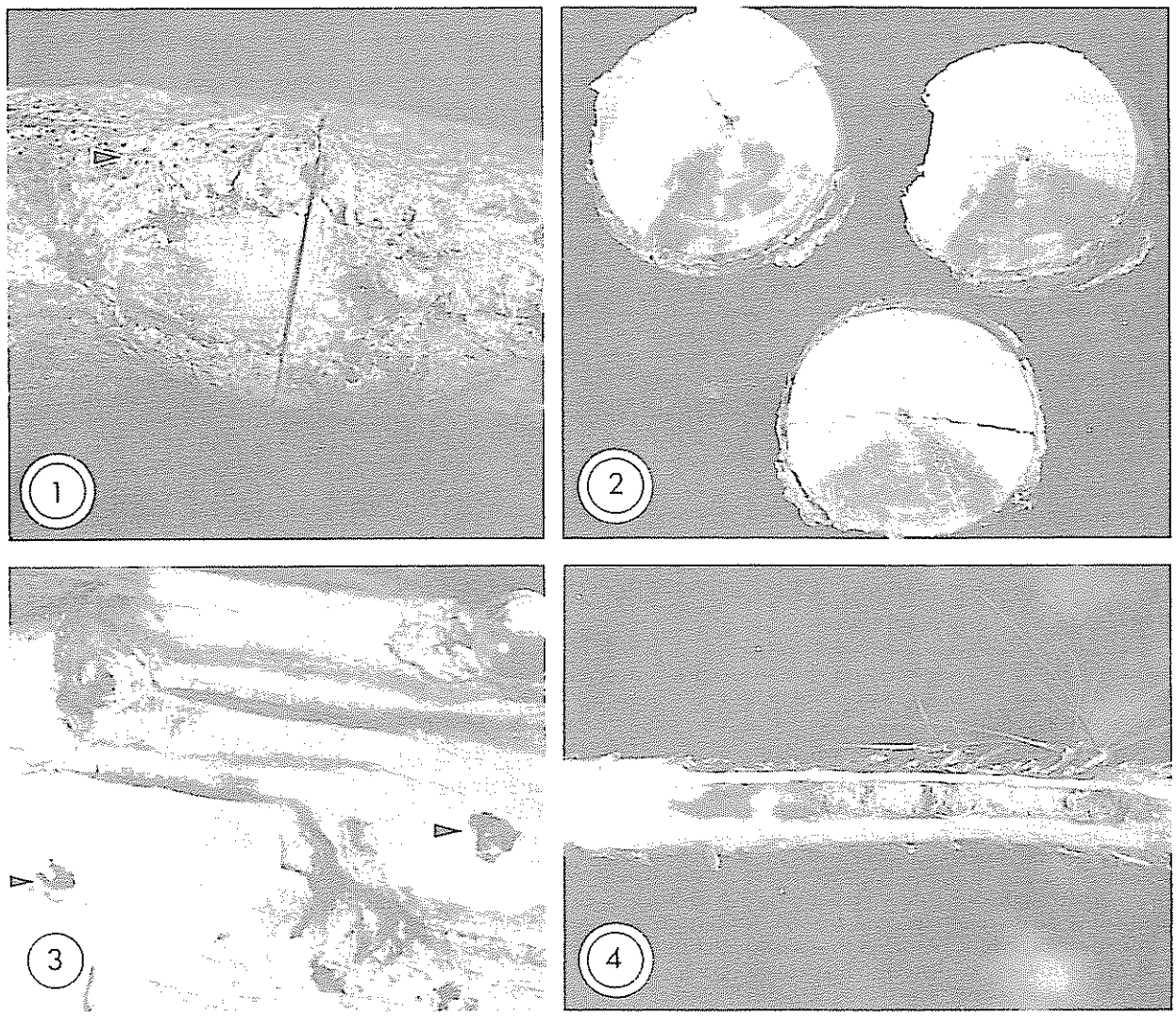

FIG. 1 Hail damaged branch of *P. radiata* with pycnidia of *S. sapinea* (arrow)

FIG. 2 Cross-section through hail wound showing wedges of *S. sapinea* infected tissue

FIG. 3 Ovipositing wounds (arrows) caused by *Pissodes nemorensis* on terminal shoot of *P. radiata*

FIG. 4 *S. sapinea* infected tissue in terminal shoot of *P. radiata* following wounding by *P. nemorensis*

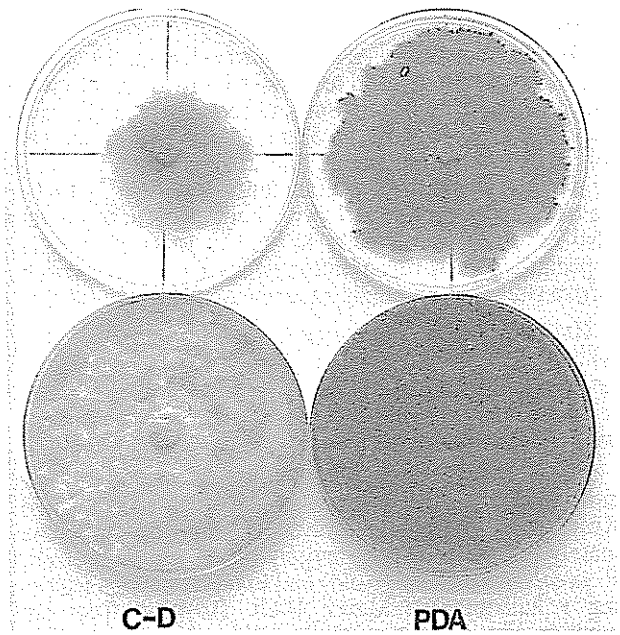

FIG. 5 Appearance of *S. sapinea* isolates PREM 48860 (top row) and PREM 48859 (bottom row) on Czapek-Dox and potato-dextrose agar after 14 d at 25 °C.

pinaster and *P. elliottii*, displayed shoot blight, dead top and resinosis following drought stress during 1985 and a hailstorm in February 1986. Of the damaged trees, 73 % consisted of pines less than 15 yr old and 27 % of pines between 15 and 35 yr old. Isolations consistently yielded *S. sapinea*. Damage was assessed at R1 605 931 (W. H. Barnard, Forestry Branch, Dept of Environment Affairs, Private Bag X12, Knysna, 6570, personal communication). No *S. sapinea* was isolated in eight cases of shoot blight and dead top associated with drought stress and hail damage from Lesotho.

The most extensive cases of shoot blight associated with drought not accompanied by hail, were those reported during January 1986 from Garcia, Swellendam and Grootvadersbosch State Forests in the South-western Cape (Table 1). Symptoms were preceded by drought during early 1985 and an above average rainfall during October, November and December of that year. No mortality was observed. Symptoms were restricted to lateral shoots and isolations from these shoots consistently yielded *S. sapinea*. At Garcia, only trees growing on shallow (0,5 m) badly drained sites with an annual increment of approximately 6 m^3/ha were diseased. Trees on sites with a higher annual increment (>10 m^3/ha) showed no disease symptoms.

Extensive dead top was observed in some compartments of *P. radiata* in the Grabouw State Forest (South-western Cape) showing signs of phosphate deficiency (D. C. Grey, Saasveld Forestry Research Centre, Private Bag X6515, George 6530, personal communication). *S. sapinea* was, however, not isolated from diseased tissue.

There were two cases of shoot blight associated with insect damage. *S. sapinea* was isolated from necrotic lesions surrounding ovipositing wounds of *Pissodes nemorensis* Germ. on dying *P. radiata* shoots (Fig. 3, 4) at Witfontein in the Southern Cape and at La Motte in the South-western Cape.

Isolates

Spore dimensions. Spores of isolates PREM 48859 and 48860 averaged 39,8 × 14,63 μm and 39,46 × 15,34 μm respectively. Spores of PREM 48860 were significantly wider (P = 0,001) than those of PREM 48859.

Effect of different media. There were significant differences (P = 0,025) in colony diameter between the two isolates on four of the media tested (Table 2). The most pronounced differences in colony diameter between isolates were on CD and CMA. Growth rates on WA were similar. The greatest radial growth of PREM 48859 was on CMA and of PREM 48860 on WA. Differences in culture morphology between isolates were most evident on CD and PDA (Fig. 5).

Effect of different temperatures. Optimum growth for both isolates occurred at 25 °C. At 35 °C very little growth was discernible after 96 h (Table 2). PREM 48860 had a significantly slower growth rate (P = 0,05) than PREM 48859 at 20, 25 and 30 °C.

Stem inoculations. There were significant differences (P = 0,01) in the amount of discolouration between the two isolates. Discolouration was more extensive on trees inoculated with PREM 48859 (Fig. 3). *S. sapinea* was isolated from lesions surrounding all wounds inoculated with the two isolates. Some discolouration also occurred in the controls, but no *S. sapinea* was recovered from these wounds.

Shoot tip inoculations. PREM 48859 infected a greater number of seedlings than PREM 48860 (Table 3). Wounded seedlings were more susceptible than unwounded seedlings to infection by both isolates. No symptoms developed on seedlings inoculated with sterile water.

DISCUSSION

Hail damage and moisture stress were associated with more than 70 % of the cases of shoot blight and dead top recorded in the surveys. Hail caused most wounds in pine trees and, under conditions favouring disease (Swart, Knox-Davies & Wingfield, 1985), was associated with the most extensive outbreaks of *S. sapinea* infection of both healthy and stressed trees. Infection associated with moisture stress alone was less extensive but more common than infection associated with hail damage. It is therefore concluded that infection is more severe when stressed trees are damaged by hail. These findings agree with those of other workers (Bega *et al.* 1978; Brown *et al.* 1981).

S. sapinea infection also occurred in the absence of mechanical wounding. The presence of shoot blight on unwounded seedlings in greenhouse inoculations was further confirmation that this fungus can infect unwounded pine shoots and colonize limited portions of host tissue. Infection of unwounded tissue was also recorded by Laughton (1937), Waterman (1943b), Marks *et al.* (1966), Brookhouser & Peterson (1971), Chou (1976a, b), and Bega *et al.* (1978).

S. sapinea was not invariably associated with dead top. Sometimes drought causes dead top in the absence of *S. sapinea* (Gibson, 1979). This was apparently the case in Lesotho where *S. sapinea* could not be isolated from dying shoots. Nutrient deficiencies such as those found at Grabouw State Forest are apparently another factor causing dead top in the absence of *S. sapinea*.

S. sapinea found in association with shoot blight and dead top does not necessarily imply that it causes the disease. It is a common saprophyte as well as an opportunistic pathogen (Swart *et al.*, 1985) and invariably colonizes dead pine tissue. For correct diagnosis of dead top and shoot blight it is therefore important to consider the involvement of other factors. This can be achieved by monitoring both site characteristics and the sequence of events such as hailstorms leading to the first appearance of symptoms.

This study provided no conclusive evidence that infection was related either to the age of trees (Peterson & Wysong, 1968; Chou, 1977; Peterson, 1981) or to the maturity of infected pine tissue (Chou, 1976b; Peterson, 1981).

The *S. sapinea* isolates in this study showed marked differences in cultural characteristics. The two isolates chosen for closer comparison also showed distinct differences in morphology and virulence. Laughton (1937) suggested that there is variation in virulence of *S. sapinea* in South Africa but conducted no pathogenicity tests. Recent reports from the USA (Wang, *et al.*, 1985; Palmer *et al.*, 1986) have shown significant variation in the virulence of different *S. sapinea* isolates. Variation in virulence could explain differences in host susceptibility and the wide range of symptoms with which *S. sapinea* is associated (Swart *et al.*, 1985). In this study, only two isolates were compared. Further studies using a wider range of isolates are required to assess the role of pathogen variation in the occurrence of *S. sapinea* diseases in South Africa.

ACKNOWLEDGEMENTS

The authors thank E. Daniels for technical assistance and S. von Broembsen, G. Dennill and M. Morris for critically reading the manuscript.

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Printed by the Government Printer, Private Bag X85, Pretoria, 0001