Seasonal Response of *Pinus radiata* in South Africa to Artificial Inoculation with *Sphaeropsis sapinea*

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ABSTRACT

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Trials were conducted in the winter rainfall region of South Africa to determine the effect of season on the rates of colonization of pine tissue by three isolates of *Sphaeropsis sapinea*. Four-year-old *Pinus radiata* trees were inoculated during fall and spring in 1986 and 1987. In both years, cambial lesions caused by *S. sapinea* on trees inoculated in spring were significantly longer than those on trees inoculated in fall. There was a significant interaction ($P < 0.01$) between seasons and isolates. Thus, season can influence the outcome of infection by *S. sapinea*. Management operations such as pruning or felling may be timed to avoid making wounds (loci of infection and colonization) before or during the summer dry seasons.

Commercial plantations of *Pinus radiata* D. Don are highly susceptible to infection by *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton in Sutton in Australia (16,17,30), New Zealand (6–8), and South Africa (12,13,25,27,28,31). Infection can result in branch or bole cankers and dieback (7,10,30), and further colonization or saprogenic activity in cut wood results in sap stain (11).

Infection often occurs through wounds caused by hail and silvicultural operations (8,10,12,13). Physiological stress due to drought or nutrient deficiencies can either predispose trees to infection or exacerbate the damage (5,18,28–30). Climatic factors also play a crucial role in the initiation and severity of infection. The dispersal of conidia of *S. sapinea* is strongly correlated with the occurrence of rain (4,26). Temperatures between 24 and 30 C and relative humidity greater than 90% are considered optimal for infection (4,6). There are conflicting reports regarding wound and stress requirements for infection by *S. sapinea* (13,16,17,25), which could be explained by the existence of different strains of the pathogen (20,24,25). It follows, therefore, that the interaction of climatic factors with those mentioned above could play a significant role in determining the outcome of infection.

In South Africa, *S. sapinea* causes considerably more damage to *P. radiata* in regions of summer rainfall where hail injury is common than in regions of winter rainfall. This has resulted in the abandonment of *P. radiata* as a commercial species in the summer rainfall region (15). Despite the sporadic occurrence of hail in the winter rainfall region of the southwestern Cape Province, there are no records of infection following hail damage (15,25). The major problems with *S. sapinea* in the winter rainfall region are the occasional infection of pruning wounds and sap stain of felled timber (25,28).

Peak dispersal times for conidia of *S. sapinea* occur during spring and summer in regions receiving winter and summer rains, respectively (26). This pattern appears to be regulated by maximum daily temperatures. Pruning and felling should, therefore, be restricted to the winter months in order to avoid periods of most abundant spore dispersal and thus, potentially, to avoid infection by *S. sapinea*. In areas that receive winter rain coinciding with moderate temperatures, however, opportunities for infection are far greater than in regions where winters are cold and dry. Furthermore, dormant trees are more susceptible to colonization by certain canker, bluestain, and decay fungi than are actively growing trees (3,9,19,23). The relative rate at which *P. radiata* is colonized by *S. sapinea* during the dormant and growing season is, therefore, crucial to the implementation of safe pruning and felling schedules in the southwestern Cape Province. This study investigated the effect of artificial inoculations with three isolates of *S. sapinea* on *P. radiata* during spring and fall in this region.

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MATERIALS AND METHODS

Inoculations were conducted in the Grabouw State Forest in the southwestern Cape Province of South Africa during April (fall) and October (spring) of 1986 and 1987. The experiment was done in an unpruned stand of 4-yr-old *P. radiata* planted 1.3 m apart. In both fall and spring, 20 trees were randomly selected and wounded on each of the four lowest lateral branches by removing a disk of bark, 10 mm in diameter and approximately 30 cm from the bole, with a cork borer. Disks of 4-day-old monoconidial cultures of *S. sapinea* isolates PREM 48859, PREM 48860, and PREM 48892 (National Collection of Fungi, Pretoria), grown on 2% malt extract agar (MEA; 20 g of Difco Bacto agar, 10 g of Difco malt extract, 1,000 ml of water), were placed in the wounds, one disk of one isolate per wound, three isolates per tree. A sterile MEA disk was placed in the fourth wound, and all wounds were covered with masking tape.

After 6 mo, bark and phloem surrounding inoculation points were removed with a scalpel and the length of lesions surrounding the inoculation points was measured. Isolations were made from discolored tissue surrounding all inoculation points. Each trial was arranged as a randomized complete block with 20 wounds per isolate. Tukey's HSD procedure for comparison of means was applied where a factorial ANOVA showed significant variation between years, seasons, and isolates.

In order to determine whether the varying thickness of inoculated branches would influence results, a separate study was done during fall and spring of the first year. Twenty trees were randomly selected in the same stand and wounded on each of two lateral branches having a diameter between 10 and 14 mm and situated in the lowest whorl. This was repeated with 20 trees having a branch diameter between 20 and 25 mm. On each tree, one wound was inoculated with a single isolate of *S. sapinea* (PREM 48859) and the other with a sterile MEA plug. A completely randomized design was used for the experiment, and Tukey's HSD procedure for comparison of means was applied after a factorial ANOVA was performed.

RESULTS

Cankers, visible as elliptical areas of sunken bark, had formed around all wounds 6 mo after inoculation. The pathogen was isolated from lesions surrounding all inoculated wounds. No branches had died as a result of girdling 6 mo after spring or fall inoculations. Control wounds showed no canker formation and very little tissue discoloration and were completely covered with callus.

Lesion length was significantly influenced by season and the different isolates of *S. sapinea*. Cambial lesions caused by isolates PREM 48859 and PREM 48892 on trees inoculated in spring were longer ($P < 0.01$) than those on trees inoculated in fall (Fig. 1). After inoculations in spring, isolate PREM 48859 caused significantly longer lesions ($P < 0.01$) than did the other two isolates and isolate PREM 48892 caused longer lesions than did isolate PREM 48860 ($P < 0.01$). There was a significant interaction ($P < 0.01$) between seasons and isolates but not between years, seasons, and isolates.

Branch diameter did not influence lesion length (Table 1). Lesions on branches of both diameter classes were significantly longer ($P < 0.01$) in spring than in fall.

DISCUSSION

The results of this study are consistent with those of artificial inoculations conducted with canker or blue-stain fungi that have been shown to cause larger cambial lesions during the growing season than during the dormant season (2,9,14,19,22). Infection of pine seedlings, young shoots, and pruning wounds by *S. sapinea* has also been reported to occur primarily during summer (4,6,8, 21). The emphasis of our study, however, was on factors affecting the extent of fungal colonization in pine tissue rather than the onset of infection.

The greater increase in cambial discoloration that we observed after inoculations in spring than after those in fall could have been influenced by factors acting either on the host or the pathogen or on their interaction. The growth of *P. radiata* in the southwestern Cape Province is strongly influenced by season. During the fall and winter months (March to August) there is a markerd reduction in the growth rate, which is followed by a flush of growth during spring (September to November) (13). Spring growth is positively correlated with rising temperatures, although its duration is determined by soil moisture, which decreases as summer approaches.

Rainfall data from the site of the experiment (Table 2) indicate that in the first and second trial, respectively, only 20 and 14% of the total rainfall for the 12-mo period spanning each trial occurred from October to March. Susceptibility of drought-stressed pine trees to infection and colonization by *S. sapinea* has frequently been reported (1,5,7,8,12,18).

Mean daily maximum temperature for the period April to September was approximately 5° higher than during the period October to March in both trials (Table 2). In Australia, higher temperatures during spring and summer were found to coincide with greater sap stain and decay activity (11). A positive correlation between mean daily temperature and lesion size over a 12-mo period after artificial inoculations with the blue-stain fungus *Ceratocystis minor* (Hedgcock) Hunt has also been reported (9).

The present study substantiates recommendations, based on spore dispersal studies, regarding pruning and felling schedules for reducing infection of *P. radiata* by *S. sapinea* in the southwestern Cape Province (26). In winter rainfall areas, months in which peak dispersal of *S. sapinea* conidia occurs (late September to November) coincide with months in which the rate of cambial colonization by *S. sapinea* is greatest. The restriction of pruning and felling to fall and winter months in winter rainfall areas should, therefore, reduce infection of pruning wounds and colonization of timber. Reduced colonization of tissue by *S. sapinea* could explain why *P. radiata* has a far lower incidence of *S. sapinea* infection in the southwestern Cape Province than in regions receiving summer rainfall, where hail injury normally coincides with warmer temperatures (12,13,25,31).

Differences in pathogenicity exist among isolates of *S. sapinea* (20,25,26). The present study substantiates this fact. Furthermore, there was a significant interaction between isolates and season of inoculation with regard to the extent of tissue colonization. Seasonal variation in the degree to which *S. sapinea* is able to colonize pine tissue should be seen in this context. Many different interactions between season, isolate variability, mechanical injury, and physiological stress are possible. Therefore, the influence of season on the virulence of *S.*

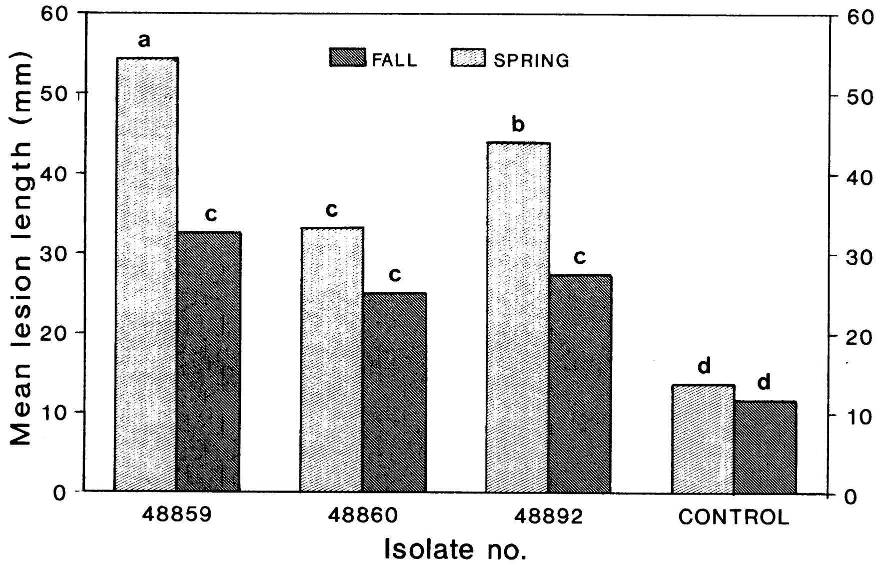

Fig. 1. Mean lengths of lesions caused by three isolates of *Sphaeropsis sapinea* after inoculations of *Pinus radiata* branches during fall and spring of 1986 and 1987. Bars designated with different letters differ significantly ($P < 0.01$) according to Tukey's HSD procedure.

Table 1. Mean length (mm) of cambial lesions on large (20–25 mm) and small (10–14 mm) diameter branches of *Pinus radiata* 6 mo after artificial inoculations in fall and spring 1986 with isolate PREM 48859 of *Sphaeropsis sapinea*

	Large diameter		Small diameter		
Treatment	**Fall**	**Spring**	**Fall**	**Spring**	**Mean**[y]
PREM 48859	31.5	66.6	36.2	72.1	51.6 a
Control	9.7	11.3	9.5	10.3	10.2 b
Mean[y]	20.6 a	38.9 b	22.9 a	41.2 b	
Mean[z]	29.8 a		32.0 a		

[y]Values followed by a different letter are significantly different at $P < 0.01$ according to Tukey's HSD procedure.

[z]Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's HSD procedure.

Table 2. Total rainfall (R_{tot}) (mm) and mean maximum (T_{max}) and minimum (T_{min}) temperatures (C) at Grabouw State Forest in the southwestern Cape Province of South Africa from April 1986 to March 1988

	April 1986–March 1987			April 1987–March 1988		
Period	T_{max}	T_{min}	R_{tot}	T_{max}	T_{min}	R_{tot}
April–September	20.8	10.5	790.1	19.8	10.0	753.7
October–March	24.8	15.5	199.8	24.9	15.4	127.7

sapinea merits further study under a wide range of site and climatic conditions.

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