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Fusarium wilt: A new disease of cultivated *Protea* in Southern Africa

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Abstract

A newly recorded disease of cultivated *Protea*, Fusarium wilt, is described and shown to be caused by *Fusarium oxysporum*. The disease occurs on mature plants (2-year-old) of *P. aristata* × *repens* cv. Venus, *P. compacta* × *susannae* cv. Pink Ice, *P. cynaroides*, *P. eximia* × *susannae* cv. Cardinal, *P. eximia* × *susannae* cv. Sylvia, *P. magnifica* × *susannae* cv. Susara and *P. repens* cv. Sneyd in the summer rainfall areas of the North-Western province of South Africa and in Zimbabwe. Disease symptoms first become visible as necrotic leaves. Subsequently, a dark lesion develops from the roots along the stem, usually visible only on one side of the stem. Occasionally the lesion develops in the upper part of the stem. The vascular tissue is discoloured leading to branch die-back and plant death. *F. oxysporum* was readily isolated from the roots, crown and vascular tissues of infected plants. Koch's postulates were proved on six *Protea* cultivars. Disease symptoms similar to those observed in the field developed 6 weeks after inoculation on all cultivars. The fungus was re-isolated from the roots, crown and vascular tissues of inoculated plants. This is the first record of Fusarium wilt on *Protea* plants.

Introduction

Cultivation of Proteaceae for export as cut-flowers is a rapidly expanding industry in South Africa, Australia, California, Hawaii, Israel, New Zealand (Forsberg 1993) and Zimbabwe (Archer 1998). In South Africa, Proteaceae are sold as fresh-cut and dried flowers; about 70% of the fresh-cut flowers and 80% of the dried flowers are exported (Wessels *et al.* 1997). *Protea* L. plants are propagated mostly as rooted and unrooted cuttings, but seedlings and seed are also used (Wessels *et al.* 1997).

In December 1997, a disease of 2-year-old *P. aristata* E. Phillips × *repens* (L.) L. cv. Venus, *P. compacta* R. Br. × *susannae* E. Phillips cv. Pink Ice, *P. eximia* (Salisb. ex Knight) Fourc. × *susannae* cv. Cardinal, *P. eximia* × *susannae* cv. Sylvia, *P. magnifica* Link × *susannae* cv. Susara and *P. repens* was observed on a *Protea* farm in the North-Western province of South Africa with an estimated incidence of 15%. Subsequently, the disease was also observed on *Protea* cultivars Pink Ice and Sylvia and also on *P. cynaroides* (L.) L. on protea farms in Zimbabwe. Other genera of the Proteaceae were also cultivated on these farms, but only *Protea* cultivars were affected. The disease was characterised by blackening of the leaves on some stems, and the development of a necrotic lesion, extending from the roots up the stem. The disease caused die-off of stems, leading to plant death. The vascular tissue within the stems of infected plants was discoloured.

A *Fusarium* sp. was consistently isolated from all symptomatic *Protea* plants. The aim of the present study, therefore, was to establish whether the *Fusarium* species isolated was the pathogen causing the wilt disease of proteas in Southern Africa.

Methods

Isolation and identification Diseased material was collected from 2-year-old *Protea* cvv. Cardinal, Pink Ice, Sneyd, Susara, Sylvia and Venus in the North-Western province, and from cvv. Pink Ice, Sylvia and *P. cynaroides* in Zimbabwe. Isolations were made from the roots, crown and vascular tissue of three plants per cultivar. Tissue was surface disinfested in 70% ethanol for 30 sec, 3% NaOCl for

1 min, and again in 70% ethanol for 15 sec. Three pieces each of the root, crown and vascular tissue were plated on potato-dextrose agar (PDA; Biolab Diagnostics, Midrand, South Africa) for each plant, and incubated at room temperature (approximately 23°C) under cool white and near-ultraviolet lights with a photoperiod of 12 h.

All fungal colonies from plated tissue were transferred to divided Petri dishes containing PDA in one half and carnation leaf agar (CLA) (Fisher *et al.* 1982) in the other half. Plates were then incubated in a growth room under near-ultraviolet and cool white light with a 12 h photo-period to induce sporulation. Single-conidial cultures were made from conidia formed on CLA; these were incubated for 3 weeks as described above . Colonies from single-conidial isolates were lyophilised. Isolates were identified according to descriptions by Nelson *et al.* (1983).

Pathogenicity tests Pathogenicity tests were conducted on 5-month-old rooted plants of cvv. Cardinal, Pink Ice, Sneyd, Susara, Sylvia and Venus. Rooted plants were transplanted into 600 mL plastic pots containing sterilised growing medium [Hygro-mix, Hygrotech, Pty (Ltd), South Africa] and inoculated 7 days later.

Inoculum and inoculation method Nine single-conidial isolates (three isolates each from root, crown and stem tissues) were used to inoculate 45 rooted plants of each cultivar (five replicates per isolate). The isolates were obtained from the same cultivars as those inoculated. Fifty-four isolates were used in total (nine isolates per cultivar, for six cultivars), and in total 270 plants were inoculated. Mycelial plugs (3 mm × 3 mm) taken from 7-day-old colonies growing on PDA were transferred to 250 mL flasks containing 100 mL of Difco potato-dextrose broth (10 g/L). Flasks were placed on a rotary shaking incubator, operating at 96 rpm at 23°C. After 5 days, the contents of each of the flasks were filtered separately through layers of sterile cheesecloth. The filtrate (mostly microconidial) was diluted with sterile distilled water to obtain inocula at a concentration of (1–10) × 10^6 microconidia per mL. A haemocytometer was used to quantify inoculum. Fifteen mL of spore suspension was pipetted around the base of each plant. Controls were inoculated with potato-dextrose broth only. The experiment was laid out in a glasshouse as a completely randomised design, with night and day temperatures of 16 and 28 °C, respectively.

Disease assessments were made 8 weeks after inoculation. Leaf necrosis, based on total leaf area per plant, was rated on a scale of 0–3, where 0 = healthy, 1 = less than 50% necrotic, 2 = more than 50% necrotic, and 3 = dead. Plants were removed from the pots and the soil carefully washed from the roots. Each plant was split open longitudinally and vascular discoloration recorded. The pathogen was re-isolated from surface-disinfested root and crown tissues. Pieces of vascular tissue were excised at 2 cm intervals along the entire length of the plant, from both sides of the stem i.e. the side of the stem with the necrotic cortical lesion and the side of the stem without an externally visible lesion. Isolations were also made from plants showing no vascular discoloration. Tissue pieces were plated on PDA and incubated at room temperature as described previously. Colonies from excised tissue pieces were identified.

Results

Isolation and identification The fungus that was isolated was identified as *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. on the basis of its morphological characteristics (Nelson *et al.* 1983). Characteristic 3-septate macroconidia with a foot-shaped basal cell and an attenuated apical cell, and single-celled microconidia borne on short monophialides were produced abundantly on CLA. Thick-walled chlamydospores, single or paired, were also observed. Colony colour ranged from peach, white-pink to purple on PDA. Bluish sclerotia were formed at the base of PDA slants and orange sporodochia were abundant on the surface of cultures. *F. oxysporum* was consistently isolated from the roots, crown and vascular tissues of each cultivar collected in the field.

Pathogenicity All cultivars inoculated with *F. oxysporum* developed severe disease symptoms similar to those observed in the field. Symptoms developed 6 weeks after inoculation. Disease symptoms were initially observed as a blackening of the leaves (Figure 1). Irregular, black patches developed on the leaves and these finally coalesced and caused the leaves to die. Necrotic leaves remained attached to the stem long after plant death. Soon after the first signs of leaf necrosis, a black lesion, visible on one side of the stem, started to develop from the soil level, extending upwards (Figure 2). The discoloured area on the stem surface correlated with

vascular discoloration inside the stem. The lesion extended from the bark, through the phloem, to the xylem tissue (Figure 3). Sometimes the lesion developed in the upper parts of the stem only. Eventually the entire plant turned black and died. The leaves did not become chlorotic and flaccid, but became stiff and dry after necrosis. *F. oxysporum* was re-isolated from vascular tissue adjacent to the necrotic cortical lesion, above the necrotic cortical lesion, and from opposite and beneath the lesion.

Isolations were also made from inoculated plants with no external symptoms. The crown and vascular tissue of the majority of these plants were discoloured and *F. oxysporum* was re-isolated from the roots, crown and vascular tissues. Control plants showed no disease symptoms and remained healthy.

Cv. Pink Ice showed the lowest percentage of leaf necrosis, lesion development, vascular discoloration, and mortality of all the inoculated cuttings (Table 1). The highest mortality rate was found in cv. Venus. Almost all the Sylvia plants developed lesions (93.3%) and there was a high percentage of vascular discoloration in both cvv. Sylvia and Venus, which were the most severely affected. Moderate symptoms were recorded for cvv. Cardinal, Sneyd and Susara (Table 1).

Discussion

Symptoms similar to those observed in the field were observed on inoculated plants in the glasshouse, and the inoculated pathogen was re-isolated, confirming Koch's postulates. Although there are reports of *Fusarium* species causing damping-off (Greenhalgh 1981; Benic 1986) and blight of propagation material of the Proteaceae (Benic 1986), this is the first record of *F. oxysporum* causing wilt of commercially cultivated *Protea* cultivars in South Africa and Zimbabwe.

F. oxysporum is one of the most important wilt pathogens and is also a common soilborne fungus that can be saprophytic or parasitic under a wide range of environmental conditions (Booth 1971). The most prominent feature of wilt pathogens is their colonisation of vascular elements, mostly the xylem, of their host (MacHardy and Beckman 1981). Although many records of Fusarium wilt caused by various formae speciales of *F. oxysporum* on woody hosts are found in the literature, including Mexican lime (Timmer 1982), grapevine (Andrade *et al.* 1993), guavas (Dwivedi 1996), Hibiscus (Gangopadhyay and Kapoor 1977) and coffee (Cardoso 1986; Negrón and Acosta 1989), few of these records include descriptions of symptoms, making comparison of the symptoms found on *Protea* difficult. In woody angiosperms, the primary symptoms include progressive foliar chlorosis, frequently accompanied by transient wilting, followed by necrosis and defoliation (Green 1981). Vascular discoloration is common to most, if not all, vascular wilt diseases of woody perennials caused by fungi (Green 1981).

The wilt disease on *Protea* is aggressive, causing leaf necrosis, the development of a black stem

Table 1 Fusarium wilt disease ratings of six *Protea* cultivars

Cultivar	Leaf necrosis (%)[A]				Percentage plants with	
	0	1	2	3	Stem lesions[B]	Vascular discoloration[C]
Cardinal	40.0	8.9	31.1	20.0	42.2	60.0
Pink Ice	77.8	0.0	15.6	6.6	20.0	22.2
Sneyd	33.3	2.3	33.3	31.1	57.8	66.7
Susara	35.6	11.1	40.0	13.3	57.8	64.4
Sylvia	6.6	15.6	37.8	40.0	93.3	95.6
Venus	22.2	6.7	20.0	51.1	80.0	80.0

[A]Percentage of inoculated plants rated according to the state of leaf necrosis: 0 = healthy; 1 = <50% necrotic; 2 = >50% necrotic; 3 = 100% necrotic.
[B]Percentage of inoculated plants that developed a necrotic lesion on the stem.
[C]Percentage of inoculated plants that showed vascular discoloration.

Figures 1–3 Fusarium wilt disease of *Protea* plants inoculated with *Fusarium oxysporum*. Diseased *Protea* cv. Sylvia (**1**) and cv. Sneyd (**2**) plants showing leaf necrosis and stem lesions. **3** Diseased *Protea* cv. Sylvia plant (left) vs control (right).


1


2


3

lesion, and vascular discoloration in the corresponding area. The symptoms on the stem of *Protea* plants closely resemble those of wilt caused by *F. oxysporum* f. sp. *chrysanthemi* Litt., Armst. & Armst. on chrysanthemum, where black stem necrosis may develop, and sometimes occurs as a streak up one side of the stem (Engelhard and Woltz 1971). On the mimosa tree, brown discoloured streaks are observed on symptomatic branches and when cut in cross section, a ring or partial ring of discoloured sapwood can be observed just under the bark in the outer wood of wilted branches (Tattar 1978). In the case of the *Protea*, the necrotic area extends from the bark, through the phloem, into the xylem tissue. On eucalypts, brown or black coloured streaks, possibly representing discoloration of the vascular tissues, are clearly visible under the bark on the basal portion of infected plants (Arya and Jain 1962).

The discoloured vascular tissue of inoculated *Protea* plants and the re-isolation of the pathogen from these tissues, indicate that the pathogen invades the vascular system. Since vascular discoloration is the most typical symptom of a wilt disease, it seems appropriate to also refer to the disease of *Protea* as a wilt. No reports of similar symptoms have been recorded on these affected cultivars in the *Protea* growing region in the South-Western Cape. Evidence thus points to this disease being limited to the summer rainfall area, where infection probably occurs during the warmer summer months. The fact that certain cultivars developed more severe symptoms than others could result from either variation in the aggressiveness of the isolates or from differences in the susceptibility of the cultivars. No cross-inoculation studies were done in the present study, however, and further research will have to be conducted to resolve this issue. The woody nature of the plants could be the reason that no typical wilt symptoms (drooping leaves of young shoots) were observed on *Protea*.

Most of the Fusarium wilt diseases are caused by formae speciales of *F. oxysporum* (Pennypacker 1981). However, in the literature root rot of pine seedlings is reported to be caused by *F. oxysporum* f. sp. *pini* (Farquhar and Peterson 1991) and root rot of coffee caused by *F. oxysporum* f. sp. *coffeae* (Dhaliwal *et al.* 1963; Anon. 1985); thus some formae speciales appear to cause root rot rather than wilt. Historically, strains of *F. oxysporum* have been divided into formae speciales on the basis of virulence on a particular host or group of hosts (Armstrong and Armstrong 1981). Further subdivisions of formae speciales into races often are made based on virulence on differential host cultivars carrying specific single dominant resistance genes (Bournival and Vallejos 1991). More recently, strains of *F. oxysporum* of various formae speciales have been grouped on the basis of vegetative compatibility (Puhalla 1985). The latter approach characterises subspecific groups on the genetics of the fungus rather than on the host-pathogen interaction (Correll 1991). Further research is therefore needed to determine the pathogenicity of the isolates obtained from *Protea* to other genera and species of the Proteaceae, such as *Leucadendron* R. Br., *Leucospermum* R. Br. and *Serruria* Salisb. and to determine whether this pathogen of *Protea* should be described as a new forma specialis of *F. oxysporum*.

Fusarium wilt disease of *Protea* has significant economic implications for crop production. Plant and branch death results, but there is also a reduction in the numbers of flowers produced by infected plants. The disease may also spread if infected plant material is used. It is therefore important that the highest level of hygiene is maintained in nurseries and that no cuttings are made from diseased material.

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