1. DIEBACK OF BLACKTHORN *(ACACIA MELLIFERA* SUBSP. *DETINENS)* IN SOUTH WEST AFRICA

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ABSTRACT

Blackthorn *(Acacia mellifera* subsp. *detinens)*, which has invaded about 10 million hectares of veld and rangeland in the northern parts of South West Africa, is subject to dieback disease which has already killed thousands of hectares of bushes and trees. Symptoms, which include leaf chlorosis, defoliation, twig and branch dieback and canker formation, are similar to those of some well-known stress-initiated dieback-decline diseases. However, the disease differs from these diseases in the formation of an internal decay of the sapwood and heartwood at the base of the trunk and upper taproot. Shoot dieback and decline was positively correlated with decay at the base of the trunk. *Phoma glomerata, P. eupyrena, P. cava* and *Cytospora chrysosperma* were consistently isolated from affected parts. The association of four different fungi with affected parts indicates that the disease could be stress-initiated.

INTRODUCTION

Acacia mellifera (Vahl) Benth. subsp. *detinens* (Burch.) Brenan, known locally as blackthorn, is a dense, more or less obconical, deciduous many-stemmed shrub or small tree (3-5 m high) with a short compact trunk branching prolifically at or above soil level. In drier parts it is encountered as a shrub 1.5-2.5 m high. This hardy, drought and heat tolerant plant, which grows well in warm, semi-arid areas with moderate to high maximum and minimum temperatures (Donaldson, 1969), is found in Tanzania and parts of southern Africa (Ross, 1979). In the northern parts of South West Africa it has invaded vast stretches of veld and rangeland often forming dense impenetrable thickets consisting of up to 10 000 bushes per hectare (Bester, 1984). Blackthorn encroachment has forced some farmers to abandon their properties (Bester, 1984).

During the mid-1970's a dieback of blackthorn was observed by farmers in parts of northern South West Africa (Bester, 1984). Little attention was given to the disease until the past 4-6 years when it reached epidemic proportions. Today about 10 million hectares are seriously affected.

As blackthorn is considered a weed in the northern parts of South West Africa, research on the etiology of this disease has become essential. This paper describes our observations on the symptomatology of this previously undescribed disease, the isolation and identification of organisms from affected tissue and its range in southern Africa in 1988.

MATERIALS AND METHODS

Disease symptoms and isolations

Observations on disease development were made over a period of years. Blackthorn bushes in various stages of symptom development were excavated during 1985-1988 and disease symptoms noted. During this period material was obtained from bushes in different localities in South West Africa, and during February 1987 in the north-western regions of the Cape Province and Transvaal. The plant material was kept in paper bags at 8±2°C until isolations were made.

The material was examined for fruiting bodies, debarked to minimize contamination, surface-disinfested for 1 min in 70% ethanol, dried and cut or split open. Small chips (3-5mm) of tissue excised from the centre and margins of discoloured parts (Fig. 1) were plated on potato-dextrose agar (PDA) (200g potato, 20g glucose, 15g agar/1) and incubated for 8-9 d at 25°C in the dark. Cultures were then kep under intermittent light (black light, 12 h cycles) to encourage sporulation. Fungi that developed from the tissue were identified, recorded and pure isolates prepared for storage on PDA slants. Isolates of *Phoma*-like fungi were cultured for identification as described by Boerema & Dorenbosch (1973), whereas *Fusarium* spp. were identified according to Nelson, Toussoun & Marasas (1983). Coelomcetes were identified according to Sutton (1980).

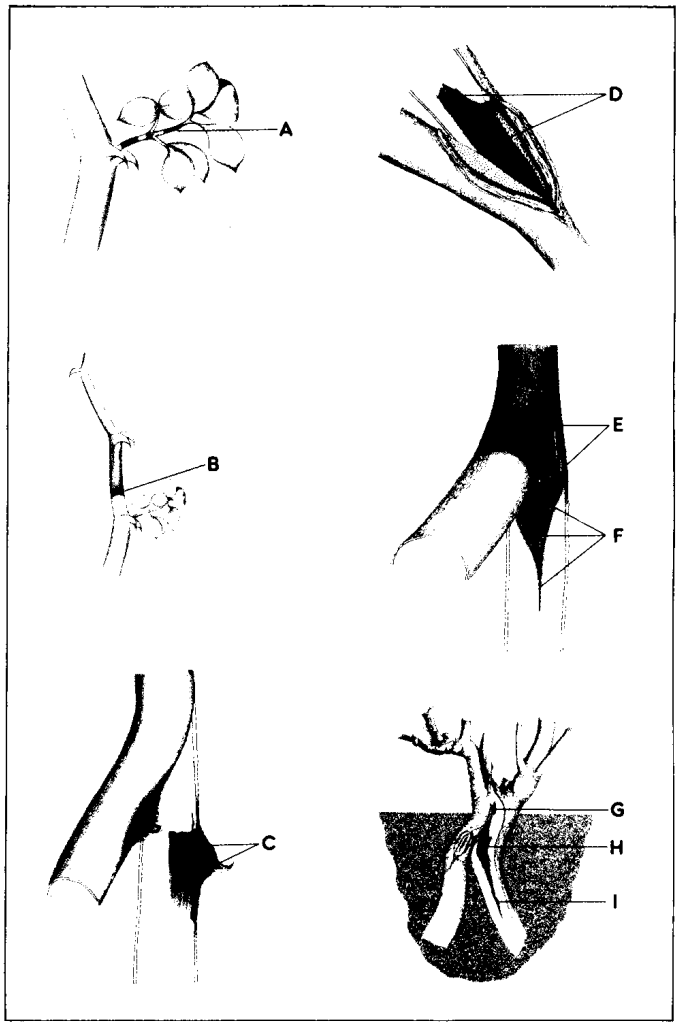

Fig. 1. Plant parts of blackthorn bushes with dieback from which isolations were made.

In addition affected tissue prepared as above was placed in moist chambers at room temperature (22±3°C) on a laboratory bench, and the appearance of fungal fruiting structures noted.

Relationship between decline and infection at the base of the trunk

Ninety-six bushes in various stages of disease development were selected and rated for dieback and decline. Twig and branch dieback were assessed on a scale of 0-10 where 0 = no dead twigs or branches evident and 10 = 91-100% of twigs and branches dead. Decline was rated on a scale of 0-10 where 0 = no twigs or branches dead and no leaf chlorosis or defoliation evident and 10 = 91-100% of the twigs and branches dead and defoliated.

Bushes were then excavated, the base of the trunks and the upper taproot were split open and examined for basal rot. Basal infection was assessed on a scale of 0-10 where 0 = no discolouration or decay evident at the stem base or upper taproot and 10 = virtually all of the sapwood and heartwood discoloured or decayed. The data was analysed statistically to estimate the correlation between above-ground symtoms and basal rot.

RESULTS

Disease symptoms

The first disease symptoms which are usually more prevalent at the end of the period of active growth (March-April), are a yellowing of leaves on individual twigs or branches, defoliation, and dieback of defoliated shoots. In some cases minute necrotic lesions occur on leaves and petioles. A black-brown discolouration, that might extend into the pith, occurs around and underneath some thorns from which the petioles had dropped. Buds in these cases were usually necrotic.

Small cankers (0.5-1cm) frequently occur on some twigs and shoots. They are longitudinal and slightly more raised than the healthy tissue. Abundant dark brown to black pycnidia are embedded in the exposed wood of most cankers, but not on the surrounding callus tissue or bark. Although cankers on actively-growing twigs have often healed, the underlying cortex may be discoloured. Long cankers are occasionally present on stems near the main trunk, and discolouration expands to the pith.

The most conspicuous symptom is an internal green-yellow to black-green discolouration and decay of wood at the base of the trunk and the upper taproot. In some older bushes or trees, no cankers or entry from the outside can be found. However, in most many-stemmed and especially young bushes, a discolouration that may extend for several centimeters downward into the upper taproot can usually be traced back to a dead stub or branch at or near soil level or, infrequently, to distinct cankers occurring approximately 20-30cm below soil level on the taproot. Other roots are unaffected.

Initially only the sapwood in the vicinity of the dead stub or canker becomes discoloured. It then expands and eventually reaches the heartwood. The distinct red-brown heartwood assumes a black-green colour, whereas the discolouration extends tangentially up or down in the compact trunk base or taproot. The heartwood and parts of the sapwood start to decay and in some plants a hollow-base condition develops with only a small portion of the xylem being functional. Dark brown to black pycnidia frequently occur in cavities formed in discoloured and decayed wood, or they are embedded in the decayed wood.

Heartwood is usually not formed at the base of the trunk of young bushes, and is sometimes not seen even in some older bushes. In such instances the older sapwood and pith become gree-yellow or black-green, and eventually form cracks in the discoloured portion.

Wood borers are occasionally found in the dead wood at an advanced stage of basal rot. Progressive death of twigs and branches in all portions of the crown then follows until the entire bush or tree dies.

Relationship between decline and infection at the trunk base

Vigorously-growing blackthorn bushes showing no dieback symptoms usually had no discolouration or wood decay at the base. No distinct cankers occurred on the upper taproot, nor were there any dead stubs at the base of the trunk. The heartwood was a distinct red-brown colour, or, when no heartwood had formed, the sapwood was unaffected. A significant positive correlation was found between above-ground symptoms (shoot dieback $r = 0.693$; decline $r = 0.716$; $P = 0.05$) and the discolouration/-decay index of the base.

Organisms isolated

The 15 955 fungi isolated from the 27 561 chips plated on PDA, and their association with specific lesions, are given in Table 1. Species of *Phoma* and *Fusarium,* and a *Cytospora* sp. were most commonly isolated. Other organisms were rarely isolated.

The *Fusarium* spp. were obtained infrequently, and were isolated singly, or in various combinations. They were identified as *F. oxysporum* Schlecht. emend. Snyd. & Hans., *F. moniliforme* Sheldon, *F. merismoides* Corda, *F. chlamydosporum* Wollenw. & Reinking and *F. scirpi* Lambotte & Fautr. The identities of the last three species were verified by W.F.O. Marasas, Medical Research Council, P.O. Box 70, Tygerberg.

The *Cytospora (C. chrysosperma* Pers. ex Fr. PREM 48927) and *Phoma* spp. were consistently isolated from plant material irrespective of the locality from which they were collected. The *Phoma* spp. were identified as *P. glomerata* (Cda) Wollenw. & Hochapf., *P. eupyrena* Sacc. and *P. cava* Schulz. Occasionally, more than one of these fungi were isolated from the same plant whereas *P. cava* and *C. chrysosperma* were frequently the only organisms recovered from the base of the trunk. The following description of the *Phoma* spp. is based on their cultural characteristics, on pycnidia and conidia formed on oatmeal agar (OMA), malt extract agar (MEA) and PDA, and also on chlamydospore characteristics.

P. glomerata. OMA: cultures variable, usually woolly, olivaceous grey to various shades of grey. Pycnidia partly immersed or superficial, solitary or coalescing, globose, subglobose, obpyriform, frequently with short or elongated necks and more than one ostiolum, dark brown or black. Wall pseudoparenchymatous with an outer layer of thick-walled brown isodiametric cells and a layer of thin-walled subhyaline cells bearing conidiogenous cells. Micropycnidia formed from aerial mycelium or arising from dictyochlamydospores are more or less globose or flask-shaped. Conidiogenous cells monophialidic, hyaline, smooth, simple, ampulliform to doliiform with a short narrow neck, mostly discrete. Conidia enteroblastic, hyaline to subhyaline, aseptate, ovoid, ellipsoid, oblong to short cylindrical, obovate, sometimes obtuse at each end. Often biguttulate or occasionally multiguttulate, 6-7.3 x 3,3-3.7 um. Single to dictyoch-

lamydospores (*Alternaria*-like), and intermediate types are formed. Dictyochlamyspores single, in branched or unbranched chains, usually individually on hyphal tips, intercalary, or in hyphal strands.

MEA: cultures variable, aerial mycelium sparse, closely appressed to medium, those with abundant aerial mycelium frequently sectoring to produce areas with sparse growth bearing numerous pycnidia. Cultures olivaceous, grey olivaceous to olivaceous grey.

PDA: cultures similar to those on MEA, but with more aerial mycelium. Cultures olivaceous grey to olivaceous buff.

P. eupyrena. OMA: cultures variable, often with dense aerial mycelium, concentrically zoned, greenish olivaceous to grey olivaceous. Pycnidia abundantly formed, concentrically zoned, partly immersed or superficial, separate or aggregated, occasionally confluent, dark brown to blackish, subglobose to obpyriform. Conidiogenous cells monophialidic, hyaline, simple, ampulliform or doliiform with a short narrow neck. Conidia enteroblastic, hyaline, aseptate, cylindrical, straight or slightly curved, occasionally biguttulate, 3.5-4.6x2-2.6 um.

MEA and PDA: cultures dull green to greenish olivaceous, dense, felty mycelium submerged at margin. Micropycnidia formed from aerial mycelium on PDA. Chlamydospores, typically formed on PDA, terminal or intercalary, often catenate, at first hyaline becoming pale, medium or dark brown. Formed abundantly in pairs or short chains.

P. cava. OMA, MEA, PDA : cultures pale olivaceous grey to olivaceous grey with dense felty aerial mycelium not concentrically zoned. Often a saffron to apricot discolouration below the colony on OMA. On PDA, cultures usually mucous in the centre and below the mycelial mat. Pycnidia abundant, arranged in narrow,

Table 1 — Incidence[a] of fungi in affected parts of blackthorn bushes with dieback obtained from different sites in South West Africa and the north-western region of the Cape Province and Transvaal:

Fungi	Incidence (%) in different plant parts[b]								
	A	B	C	D	E	F	G	H	I
Phoma glomerata	100	100	79	70	95	32	20	6	6
P. eupyrena	0	12	34	27	20	24	40	41	6
Fusarium spp.[c]	0	6	3	15	20	6	55	24	14
Cytospora chrysosperma	0	0	3	9	0	0	35	35	44
P. cava	0	0	10	12	3	4	5	12	25
Haplosporella sp.	0	0	10	0	20	10	10	0	3
Diplodia mutila	0	6	3	0	7	10	0	6	0
Sphaeropsis sp.	0	0	7	0	0	5	5	0	0
Melanophoma karoo	0	6	0	0	10	6	0	0	0
Phomopsis sp.	0	0	0	6	5	0	0	6	0
Other fungi[d]	0	0	5	8	2	5	2	1	1

[a]Isolated singly, or in combination from 15 955 out of 27 561 chips of tissue.

[b]For plant parts see Fig. 1.

[c]Incidence for *F. oxysporum, F. moniliforme, F. merismoides, F. chlamydosporum* and *F. scirpi.*

[d]Incidence for species of *Coniothyrium, Pencillium, Aspergillus, Camarosporium, Tryblidiopycnis, Neocosmospora, Fusicoccum, Stagonospora* and other unidentified fungi.

Table 2 — Predominant fungi based on frequency of isolation from affected parts of blackthorn bushes with dieback obtained from different sites in South West Africa and the north-western region of the Cape Province and Transvaal:

Fungi	Relative frequency (% of total) in affected plant parts[a]								
	A	B	C	D	E	F	G	H	I
Phoma glomerata	100.0	100.0	63.0	51.4	50.0	41.7	0	0	0
P. eupyrena	0	0	37.0	22.9	50.0	27,1	27.3	31.6	19.2
Cytospora chrysosperma	0	0	0	5.7	0	0	36.4	21.1	46.2
P. cava	0	0	0	11.4	0	0	13.7	15.8	19.2
Fusarium spp.[b]	0	0	0	8.6	0	2.1	13.6	10.5	11.5
Haplosporella sp.	0	0	0	0	0	10.4	0	0	3.9
Diplodia mutila	0	0	0	0	0	6.3	0	5.3	0
Phomopsis sp.	0	0	0	0	0	0	0	10.5	0
Other fungi[c]	0	0	0	0	0	12.4	9.0	5.2	0

[abc]See Table 1.

more or less concentric circles, doliiform to papillate, dark brown to black, usually separate and thin-walled. Opening of circular ostioles surrounded by hyaline cells. Conidia originate from discrete ampulliform, obpyriform to obclavate conidiogenous cells and from branched filiform conidiophores. On condiophores integrated conidiogenous loci occur below transverse septa. Chlamydospores unicellular, intercalary, pale to dark brown, formed on hyphal tips or in chains. Occasionally single and pgrahmospore-like in water mounts. Conidia enteroblastic, hyaline, aseptate, cylindrical or ellipsoid, straight or clightly curved, occasionally tapered at one end, biguttulate, 2.4-3.8x1.5-2.0 um.

The predominant fungi in affected plant parts, based on frequency of isolation, are given in Table 2. According to this criterion *P. glomerata* was the predominant fungus isolated from petioles and discoloured wood underneath thorns, and, with *P. eupyrena* the predominant fungi isolated from cankers and discoloured wood in twigs and branches. *P. eupyrena, P. cava* and *C. chrysosperma* predominated in necrotic tissue from the base.

Pycnidia of *P. cava, P. eupyrena* and *P. glomerata* predominated the exposed cankers. Those of *C. chrysosperma*, other unidentified *Phoma, Phomopsis, Camarosporium, Stagonospora* and *Diplodia* spp. occurred less commonly. Perithecia of *Pleospora* were occasionally seen in cavities in decayed wood. When placed in moist chambers, pycnidia and chlamydospores of *P. glomerata* formed abundantly on lesions on petioles and leaves, the base of discoloured thorns, bark on dead twigs and branches, and, even after rigorous surface disinfestation, on the bark of healthy material obtained from affected plants.

Borers found in the wood were of the families Buprestidae and Cerambycidae (identified by Prof. G. Scholtz, University of Pretoria). These borers occurred only in affected wood, never in sound, unaffected parts.

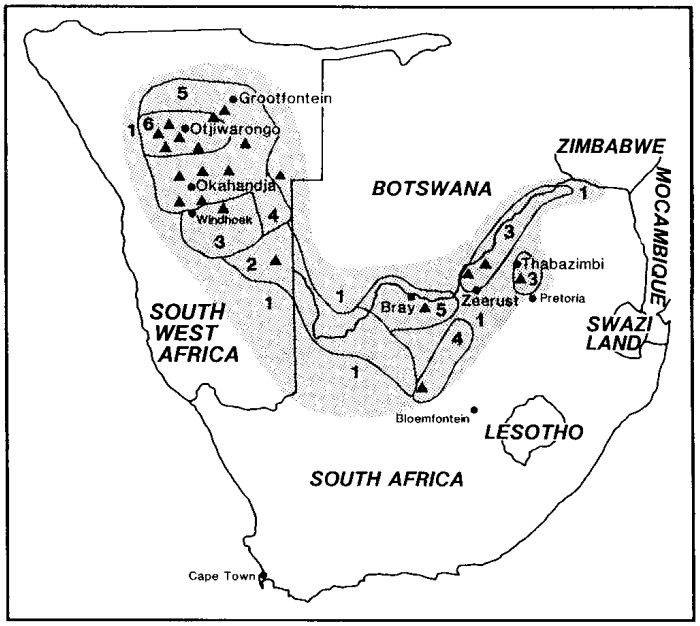

Fig. 2. Localities in South West Africa and the northwestern region of the Cape Province and Transvaal where blackthorn dieback was confirmed by the identification of conidiomata and/or isolation of *P. glomerata, P. cava, P. eupyrena* and *C. chrysosperma* from wood of the base of the trunk or upper taproot. Values 1-6 indicate encroachment of the different regions of blackthorn: 1 = less than 1 000 bushes/ha; 2 = 1 000-3 000 bushes/ha; 3 = 3 000 5 000 bushes/ha; 4 = 5 000-7 000 bushes/ha; 5 = 7 000-9 000 bushes/ha; 6 = more than 9 000 bushes/ha.

Disease range

Fig. 2 shows the approximate range of blackthorn dieback in the northern parts of South West Africa and the Republic of South Africa. It was based on a survey of disease symptoms and was confirmed by the identification of conidiomata and/or isolation of *glomerata, P. cava, P. eupyrena* and *C. chrysosperma* from wood of the trunk base and taproot of affected bushes or trees. The disease was found in all the regions invaded by blackthorn and also in uninvaded areas. Thousands of hectares of dead blackthorn occur in the Hochfeld, Omatako, Otjiwarongo and Windhoek regions, whereas bushes or trees showing various stages of dieback were observed throughout the northern parts of the country. Blackthorn dieback was also identified at sites in the Republic of South Africa (Molopo, Zeerust and Thabazimbi regions). However, no patches of dead bushes or trees were seen

DISCUSSION

Observations on disease development made during 1985-1988 revealed features typical of an infectious biotic disease. Bushes of all sizes and ages growing alone or in thickets and in stands growing different habitats or soil types were infected. Individual trees started to die back, and eventually formed patches of dead bushes in which other indigenous vegetation remained unaffected. Patches enlarged until several hectares of blackthorn bushes had died. No recovery of diseased bushes in dead patches has been observed.

From fructifications on necrotic tissue, isolations from infected petioles, twigs branches and trunk bases, we have concluded that *P. glomerata, P. cava, P. eupyrena* and *C. chrysosperma* are the primary causal organisms of dieback of blackthorn. The association of four different pathogens with dieback indicates that the disease could be similar in etiology to other stress-initiated dieback-decline diseases, which are nearly always associated with a stress factor and organisms of "secondary action " (Houston, 1973, 1987).

Species of *Cytospora* have previously been associated with stress-initiated canker or dieback-decline diseases (Long, 1918; Schreiner, 1931; Bertrand *et al.*, 1976, Domanski, 1983), whereas the *Phoma* spp. cause severe diseases on other hosts. *P. eupyrena* has been associated with blight and needle cast of red fir and Douglas-fir (Kliejunas *et al.*, 1985), *P. apopsphaeriodes (= cava)* with canker of oak (Carter, 1941) and *P. glomerata*, although considered to be a weak parasite and secondary invader (White & Morgan-Jones, 1987) with dieback of grapevines (Granata & Refatti, 1981) and pears (Chohan & Chand, 1980).

In some respects the diesease of blackthorn differs from other stress-related diebacks and declines. Contrary to ash dieback and beech bark disease (Houston *et al.*, 1979), there were no large stem and branch cankers or decayed bark that could be responsible for the death of large portions of the crown. Neither were any root infections observed, as with beech bark disease (Houston, 1973), decline diseases of maple and oak (Houston, 1973; Manion, 1981) and pole blight of pine (Manion, 1981). In the case of blackthorn, no tufts of sprout-origin foliage, coppice shoot formation or a rapid dieback of terminals, phenomena most common to other stress-initiated declines (Houston, 1973), were observed during the 4-year period of study. Instead, dieback follows decay of heartwood and extensive parts of sapwood at the base of the trunk and upper taproot, rather than the action of these organisms on branches or stems.

It is therefore postulated that the progressive decay causes a gradual cut-off of water and nutrient supply to the crown, followed by a dieback and decline of the entire bush or tree.

The reason for the sudden onset of blackthorn dieback over part of South West Africa is unknown. We suggest that the disease was present in the northern parts of the country before the recent outbreak but at a low level of infection. Abiotic factors favouring a significant increase in infection could have led to a rapid build-up of inoculum, thereby perpetuating new cycles of infection. This is confirmed by farmers who noticed similar symptoms of dieback in the Hochfeld area during the mid 1970's. On these farms, which were heavily infested, virtually all the bushes died during the period 1985-1988, leaving open veld.

Stress as defined by Houston (1973), can be either abiotic or biotic. In South West Africa rainfall is characterised by great spatial and temporal variability (Katsiambirtas 1988) and drought and heat stress are common. However, blackthorn is considered as a drought-tolerant plant well adapted to the climatic conditions of the territory (Donaldson, 1969). Therefore. the primary initiating stress factor(s) and the pressure exerted by them on blackthorn remain obscure.

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