Blight of Pincushions (*Leucospermum* spp.) Caused by *Drechslera dematioidea*

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ABSTRACT

von Broembsen, S. L. 1986. Blight of pincushions (*Leucospermum* spp.) caused by *Drechslera dematioidea*. Plant Disease 70:33-36.

A new disease of cultivated pincushions (*Leucospermum* spp.), pincushion blight, is described and shown to be caused by *Drechslera dematioidea*. The disease occurs in the major pincushion production areas of the southwestern Cape Province. Direct crop losses result when blight occurs on flower heads or flower-bearing shoots. Among 10 species, four hybrids, and eight cultivars of *Leucospermum* recorded as susceptible, *L. cordifolium* and cultivars derived from *L. cordifolium* are the most severely affected. The disease also occurs on pincushions in their native habitat. *Botryosphaeria dothidea* is shown to be an important secondary pathogen of the stem cankers caused by *D. dematioidea*.

Cultivation of proteas (Proteaceae) for export as cut flowers is a young but rapidly expanding industry in South Africa. These unique cut flowers are exported primarily to Europe, where high prices are obtained. Pincushions (*Leucospermum* spp.) are a main component of this export industry (5). All 48 known *Leucospermum* spp. are indigenous to South Africa (7). Of these, only a few species are cultivated on a significant scale; *L. cordifolium* (Salisb. ex Knight) Fourc. is the most important and most widely cultivated species. Vegetative reproduction of pincushion selections and cultivars is still a relatively new practice. About 20 pincushion cultivars have been developed, primarily from *L. cordifolium* selections and hybrids. In addition, pincushions are often cultivated from genetically diverse seed.

In 1978, a disease of pincushions that caused a severe blight of current-season leaves, stems, and flower heads was observed in several commercial plantings in the southwestern Cape Province. This paper describes the symptoms, pathogen, and effects on crop production of this new disease and offers some suggestions for its control.

Accepted for publication 16 January 1985.

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MATERIALS AND METHODS

Field observations and sampling. Samples of affected foliage, stems, and flower heads were taken from diseased pincushions in commercial plantings and in native habitats in the southwestern Cape Province. Blight symptoms were recorded on 38 pincushion selections and cultivars in breeding trials of the Horticultural Research Institute at Tygerhoek Experimental Farm, Riviersonderend, during 1983–1984.

Isolation and identification. Diseased plant parts were incubated in moist chambers to induce sporulation, then hand-sectioned and mounted in lactophenol for microscopic examination. Isolations were made from symptomatic tissues on malt-extract agar (MEA) (10 g of Difco malt extract and 20 g of Difco-Bacto agar per liter). Fungal isolates were cultured on potato-carrot agar (PCA) (150 g of potato, 30 g of carrot, and 20 g of Difco-Bacto agar per liter) at 18–20 C in natural light on a laboratory bench to induce sporulation.

Pathogenicity tests. Conidial inoculum was prepared by growing single-spore *Drechslera* isolate R26 (PREM 47341, National Mycological Herbarium, Pretoria) on PCA for 14 days at 18–20 C in natural light. Conidial suspensions in sterile distilled water were adjusted to 1 × 10^4 conidia per milliliter. Foliage of 20 6-mo-old plants of the pincushion cultivar *L. cordifolium* 'Gold Dust' was atomized with the conidial suspension. Ten similar control plants were sprayed with sterile distilled water. All plants were covered with clear plastic bags for 24 hr and maintained in a growth chamber at 16–20 C with indirect lighting from adjacent light banks on a light/dark cycle of 8/16 hr. Symptom development was observed and recorded. Isolations were made from all plants after 6 wk.

Field inoculations. Inoculum was prepared by growing *Drechslera* isolate R2G1 (PREM 45957) on sterile wooden toothpicks on 20% clarified V-8 juice agar in petri dishes and incubating at 24 C for 15 days. Toothpick inoculum of a *Botryosphaeria* isolate (R2G6B) from a blight canker on a Gold Dust stem was similarly prepared. Toothpicks from uninoculated plates were used for control inoculations. A trial was laid out in a field of 3-yr-old Gold Dust plants in a randomized block design. Five treatments (Table 1), with five replicates each, were randomly distributed within blocks; each block was repeated five times. Current-season stems and developing flower heads were inoculated with either one or both fungi by placing the pointed ends of half toothpicks in wounds made with sterile forceps. When both fungi were inoculated together, the toothpicks were placed 1 cm apart across the stem. All lesions were measured after 12 wk. Isolations on MEA were made from all treatments.

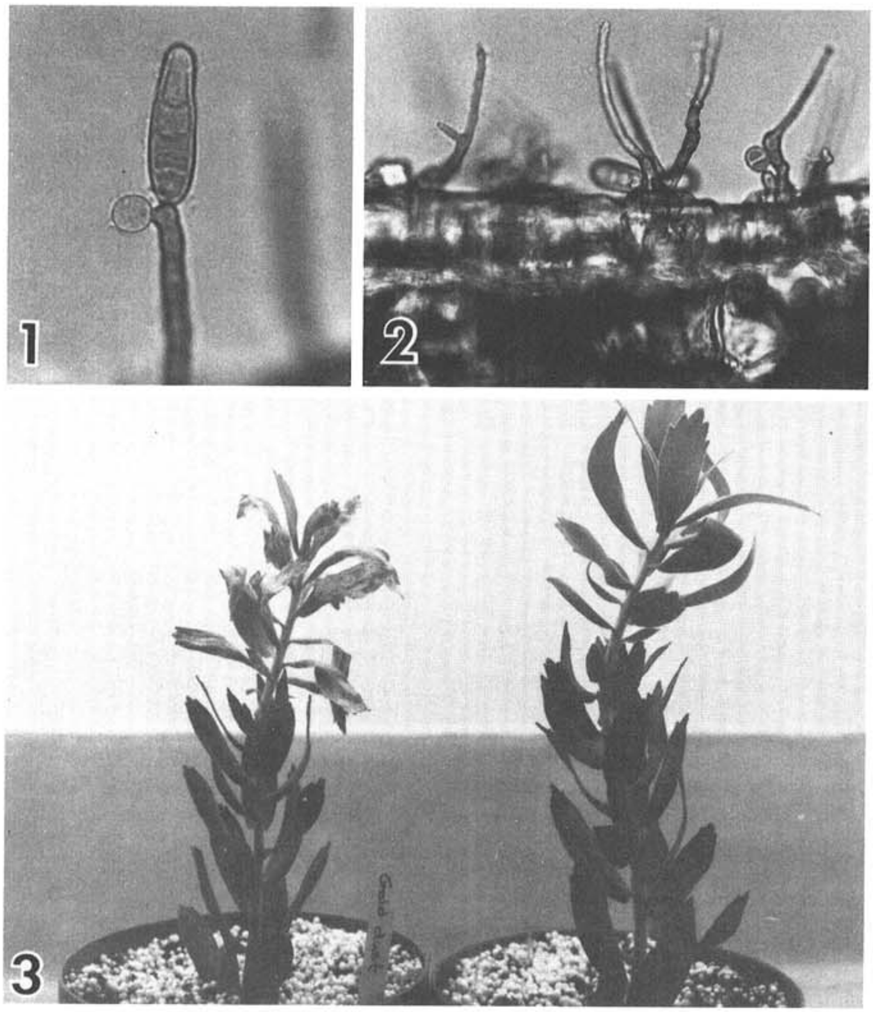
Table 1. Field inoculations of *Leucospermum cordifolium* 'Gold Dust' with *Drechslera dematioidea* and *Botryosphaeria dothidea*

Inoculated plant part	Fungus inoculated	Percent recovery from cankers		Mean canker length[y] (cm)
		D. dematioidea	***B. dothidea***	
Stem	*D. dematioidea*	100	8	5.4 a[z]
	B. dothidea	0	100	1.9 b
	D. dematioidea + *B. dothidea*	100	96	6.2 c
Flower head	*D. dematioidea*	100	8	1.9 b
Controls	None	0	25	0.7 d

[y] Average calculated from 25 measurements. Stem cankers were measured longitudinally. Flower head cankers were measured latitudinally, so canker length was limited by flower head circumference.

[z] Means followed by the same letter do not differ significantly (LSD, $P = 0.05$).

Isolation of fungi from seeds. Thirty flower heads on Gold Dust plants with blight symptoms were marked for collection after maturation. Seeds removed from these flower heads were surface-disinfested for 2 min in 1% NaOCl, rinsed in sterile distilled water, blotted on sterile paper towels, and plated on MEA (three seeds per plate). Plates were incubated at 18–20 C in natural light. Isolates from developing colonies were cultured on separate plates of MEA and PCA for identification.



Figs. 1-3. (1) *Drechslera dematioidea* conidiophore with developing conidia. **(2)** *D. dematioidea* conidiophores protruding from the surface of a pincushion leaf. **(3)** (Left) Inoculated pincushion with blight symptoms on new foliage and (right) healthy control pincushion.

RESULTS

Field symptoms and occurrence. Severe, epidemic blight of pincushion foliage occurred primarily during the late winter to mid-spring (August–October). The weather during this period is characterized by frequent rainfall or heavy dew, morning fog, and moderate midday temperatures that can reach 20–25 C. Under these conditions, symptoms developed rapidly and most plants in a field became diseased. On leaves, symptoms appeared as yellow, irregularly shaped lesions that enlarged rapidly, often covering the entire leaf. These lesions were first light brown, then a darker gray-brown as they became necrotic. On the shoots, bright red cankers developed on the bark. Extensive canker development caused shoot death. Young vegetatively produced plants with predominantly current foliage and seedling plants in the juvenile stage with continuously growing foliage were more severely affected and sometimes died. On developing flower heads, light brown lesions initiating from a single bract enlarged rapidly, resulting in wilting and premature death of the flower heads.

Symptoms were, however, usually milder and less extensive than those described. Blight commonly occurred either as discrete leaf lesions with distinct red margins and slowly expanding stem cankers or as rapidly expanding lesions that were limited to a few leaves or to leaf tips only. These mild symptoms were observed on occasional individual plants within severely blighted fields and on pincushions in their native habitat.

Pincushion blight was recorded in production areas near Paarl, Stellenbosch, Somerset West, Grabouw, Kleinmond, Bot River, and Bredasdorp, on pincushion selections and cultivars at the Tygerhoek Experimental Farm, Riviersonderend, and on plants from several nurseries. The disease was also observed on naturally occurring *L. conocarpodendron* in the Cape Point Nature Reserve and near Somerset West, Sir Lowry's Pass, Kleinmond, and Betty's Bay as well as on *L. cordifolium* in the Houhoek Pass area.

Blight of cultivated pincushions was recorded on *L. conocarpodendron* (L.) Buek, *L. cordifolium*, *L. cuneiforme* (Burm. f.) Rourke, *L. erubescens* Rourke, *L. glabrum* Phill., *L. grandiflorum* (Salisb.) R. Br., *L. patersonii* Phill., *L. pluridens* Rourke, *L. tottum* (L.) R. Br., and *L. vestitum* (Lam.) Rourke; on hybrids of *L. cordifolium* × *L. tottum*, *L. cordifolium* × *L. lineare* R. Br., *L. conocarpodendron* × *L. cuneiforme*, and *L. tottum* × *L. vestitum*; on the *L. cordifolium* cultivars Gold Dust, Yellow Bird, Fire Dance, Vlam, and Flame Spike; on the hybrid cultivars Red Sunset (*L. cordifolium* × *L. lineare*) and Caroline (*L. cordifolium* × *L. tottum*); and on the *L. glabrum* cultivar Helderfontein. The most severely affected species was *L. cordifolium* and the most severely affected cultivars were the *L. cordifolium* cultivars Gold Dust, Yellow Bird, and Fire Dance.

Isolation and identification. A fungus identified as *Drechslera dematioidea* (Bubak & Wroblewski) Subram. & Jain on the basis of conidial characteristics (1,2,4) (Fig. 1) was consistently isolated from lesion tissues and was observed in these tissues after moist-chamber incubation (Fig. 2). Production of characteristic 3–5 septate conidia was abundant on PCA after 10–14 days of incubation at 18–20 C under natural light. Predominately nonlateral, bipolar germination was observed for several isolates. Distinctive networks of dark-brown stromatal initials and anastamosing hyphae developed in older MEA cultures. *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (=*B. ribis* Grossenb. & Dug.) was frequently isolated along with *D. dematioidea* from older, darker stem cankers.

Pathogenicity. All plants inoculated with *D. dematioidea* developed severe blight symptoms similar to those observed in the field. Light yellow lesions developed on leaves and stems 3–5 days after inoculation. They enlarged rapidly and turned orange and later brown. Only new growth was affected (Fig. 3). *D. dematioidea* was reisolated from all inoculated plants. Control plants showed no symptoms and the fungus was not isolated from them.

Field inoculations. *D. dematioidea* and *B. dothidea* were recovered from all cankers that developed from wounds into which they were inoculated (Table 1). Cankers from *D. dematioidea* inoculations were red and superficial, whereas cankers from *B. dothidea* were dark brown-black and sunken. Cankers developed from 25% of the control wounds. *D. dematioidea* was never isolated from these cankers, but *B. dothidea* was recovered from all cankers developing from control wounds. The cultural morphology of these isolates was diverse and differed from that of *B. dothidea* isolate R2G6B, used as inoculum in the trial. Canker development of all inoculated treatments was significantly greater than that of the controls (Table 1). *D. dematioidea* caused significantly larger lesions than *B. dothidea* by the end of the 12-wk trial. Simultaneous inoculation with both fungi resulted in significantly larger lesions than with either alone.

Fungi isolated from seeds. Because of heavy predation by birds and rodents, only 53 seeds were recovered from the 30 Gold Dust flower heads marked for collection from diseased plants. *D. dematioidea* was recovered from only one seed, but *B. dothidea* was recovered from 32 seeds.

DISCUSSION

D. dematioidea is the primary pathogen causing pincushion blight, a previously undescribed disease of commercially cultivated pincushions in South Africa. Pincushion isolates are considered to be *D. dematioidea* because they fit the description of isolates previously recorded in South Africa on lawn grasses (several *Cynodon* spp.) and wheat (*Triticum* sp.) as *Helminthosporium dematioideum* Bubak & Wroblewski (4). As a consequence of the creation of the genus *Drechslera* for those *Helminthosporium* spp. occurring on graminicolous hosts (2), *H. dematioideum* has been reduced to synonomy with *D. dematioidea*. However, *D. dematioidea* is known as a saprophyte or weak parasite of grasses (2,4), whereas the pincushion fungus is a virulent pathogen of a completely unrelated host. The germ tube position and type of germination of pincushion isolates also differed from that reported for *D. dematioidea* (1). Consequently, further examination of isolates from *Leucospermum* spp. and comparative studies with authenticated isolates of *D. dematioidea* are in progress. *B. dothidea* is shown here to be a wound pathogen of pincushions and an important secondary pathogen of the stem cankers caused by *D. dematioidea*. The presence of *B. dothidea* in 25% of control wounds in the field inoculation trial and on more than 60% of the tested seed indicates that natural field inoculum levels are high.

The prevalence of pincushion blight and the economic losses it causes to pincushion production have not yet been determined. This study shows, however, that the disease is widely distributed in production areas and can have economically significant effects on crop production. Direct losses of the flower crop through death of flower heads and flower-bearing shoots occur. Damage to foliage greatly reduces the market value of the crop. When shoot growth that would normally bear the following year's crop dies, additional shoots are usually produced, but these shoots are often too short to be marketable because of the reduced growth period. Seedling plants in the juvenile stage and young vegetatively produced plants with predominantly new foliage can be lost before they begin to bear flowers. In fields where epidemics have occurred, higher inoculum levels resulting from persistent stem cankers can also increase the chance of further economic loss.

Commercially cultivated pincushions appear to be more severely affected by pincushion blight than pincushions in their natural habitat. This is probably due to factors inherent in horticultural practices. Firstly, monoculture places a greater number of susceptible plants closer to each other than would occur in nature. This effect is further enhanced by the use of vegetatively produced cultivars, which provides more syncronized growth of susceptible shoots than with seed-grown plants. Harvesting of flowers causes pincushions to begin shoot growth earlier than would occur naturally (G. Jacobs, Department of Horticulture, University of Stellenbosch, *personal communication*) and therefore causes shoot growth during the period of optimum weather conditions for disease. The relatively new practice of pruning 1-yr-old plants in August to force long shoot growth (3) has the similar effect of causing shoot growth during the optimum disease period. The most severe examples of blight were observed at sites that were low-lying, along rivers or the sea, on the shady sides of windbreaks, or in the morning shadows of mountains. Planting in areas where dew, fog, or other moisture is retained until midmorning to late morning thus seems to favor pincushion blight.

Several observations suggest that control of pincushion blight through the development of resistant cultivars may be possible. In severely blighted fields grown from seed, most plants show the severe symptoms. Those individual plants that do not, however, often have smaller lesions with distinct red margins, fewer lesions per plant, or both. The marked susceptibility of the popular, well-established cultivar Gold Dust that has forced its abandonment indicates that major differences in susceptibility exist among cultivars and that selection for resistance is possible. The wide range in blight severity observed on cultivars and selections in breeding trials of the Horticultural Research Institute at Riviersonderend included complete absence of symptoms. No symptoms occurred on any replicates of certain lines despite conducive climatic conditions and high inoculum levels. A program to evaluate the susceptibility of breeding lines is under way.

Control of pincushion blight by fungicides may also be possible. In preliminary field trials (S. L. Von Broembsen, *unpublished*), iprodione, which is effective against *Helminthosporium*-type diseases, reduced disease severity in the field. Furthermore, because both *D. dematioidea* and *B. dothidea* were isolated from seeds, seed treatment appears to be advisable, particularly when seed is planted in areas where *D. dematioidea* is not known to occur.

The distribution and the host range of *D. dematioidea* in South Africa is unknown, but its presence on pincushions in their native habitat suggests that it is indigenous to this region. *Phytophthora cinnamomi* Rands, which causes an important root disease of pincushions (5), also appears to be indigenous (6). The local native vegetation is the natural home not only of many different indigenous proteas but also of a wide array of their natural pests and pathogens, many of which have not yet been studied or named. As horticultural production of proteas replaces picking these flowers from the natural vegetation, new disease and pest problems can therefore be expected to arise from cultivation of proteas in their native habitat. Conversely, cultivation of South African proteas abroad should not be plagued by these native diseases and pests if control is exercised when propagation material, including seed, is introduced to overseas production areas.

ACKNOWLEDGMENTS

I thank G. C. A. van der Westhuizen for identification of *Drechslera dematioidea* cultures, W. F. O. Marasas for identification of *Botryosphaeria dothidea* cultures, G. Jacobs for Gold Dust material and useful discussions on pincushion production, the S.A. Nature Foundation for use of the field site at Protea Heights, G. J. Brits for access to and information on unreleased breeding lines, and P. S. Knox-Davies for assistance with manuscript preparation.

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